INFECTIOUS COMPLICATIONS IN THE SOUTH AFRICAN BLACK CHILD WITH CANCER

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctorate of Philosophy in the branch of Medicine.

Johannesburg 2016

DECLARATION

I, Gita Naidu, hereby declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Gita Naidu

31 October 2016

DEDICATION

To my sunshine, my moonshine and my starshine

Christian, Carl, and Anil

PRESENTATIONS

1. G Naidu, L Wainwright, S Poyiadjis, D Mackinnon, B Rowe, SA Madhi
   Antimicrobial Susceptibility of Gram-Negative and Gram-Positive Bacteria in Children treated for Haematological Malignancies
   8th World Society for Paediatric Infectious Diseases Congress, Cape Town, South Africa: 19-22 November 2013; Abstract number: P-182, Poster Presentation

2. G Naidu, L Wainwright, S Poyiadjis, D MacKinnon, B Rowe, SA Madhi
   Infectious Complications in HIV-Infected and HIV-Uninfected Patients treated for Non-Hodgkin's Lymphoma
   The International Society of Paediatric Oncology, Africa Meeting 2012, Cape Town, South Africa: 21-23 March, 2012; Abstract number: O-57, Oral Presentation

3. G Naidu, L Wainwright, S Poyiadjis, D MacKinnon, B Rowe, A Izu, S A Madhi
   Bacterial Infections in Children Treated for Haematological Malignancy
   47th Congress of the International Society of Paediatric Oncology, Cape Town, South Africa: October 8-11, 2015; Poster Presentation

4. G Naidu, L Wainwright, S Poyiadjis, D MacKinnon, B Rowe, A Izu, SA Madhi
   Respiratory Viruses, a common Microbiological finding in Children treated for Cancer in South Africa
   47th Congress of the International Society of Paediatric Oncology, Cape Town, South Africa: October 8-11, 2015; Poster Presentation

5. G Naidu, L Wainwright, S Poyiadjis, D MacKinnon, B Rowe, A Izu, SA Madhi
   Viral and Bacterial Co-Infections in Children treated for Cancer
   47th Congress of the International Society of Paediatric Oncology, Cape Town, South Africa: October 8-11, 2015; Poster Presentation

ABSTRACT

Introduction: The cure rates for children with cancer have improved with the use of high-intensity chemotherapy, aggressive surgical techniques, radiotherapy, and bone marrow transplantation. These treatment modalities render them more susceptible to immunosuppression and increase their susceptibility to infectious complications, which is a leading cause of death in this group of patients. Children diagnosed with cancer additionally are immunocompromised due to underlying malnutrition, HIV-infection, and *Mycobacterium tuberculosis* infection, which could further compound the increased susceptibility to infectious complications.

Objectives: The aim of this research program was to investigate the infectious related morbidity and mortality in predominantly black-African children treated for cancer at one of the largest hospitals in the Southern Hemisphere (Johannesburg, South Africa) and in a setting with a high prevalence of HIV-infection and tuberculosis.

Methods: The events studied were all febrile episodes in children who received cancer treatment. The standard care for these patients included a detailed history and physical examination, blood investigations (full blood count with a differential and a blood culture for suspected sepsis). In addition, patients enrolled into the study had anthropometric measures undertaken on admission, a tuberculin skin test by the Mantoux method and blood was drawn to undertake the T.-SPOT.TB test. For each septic episode, a detailed history and physical examination was performed, blood was obtained for culture, full blood count and differential, CRP and PCT, a nasopharyngeal aspirate was performed for the identification of respiratory viruses, and empiric antibiotic treatment was initiated.

Results: We documented a high incidence (per 100 child years) of suspected septic episodes (102.9), microbiologically confirmed sepsis (69.4), respiratory virus associated (61), and 27.4% of all microbiologically confirmed septic episodes had respiratory viral and bacterial/fungal co-infections. In addition, a high proportion of septic episodes were polymicrobial in aetiology (40.6%). We also documented an incidence of 4.7 per 100 child

years of tuberculosis in our study cohort. All of these were higher in the cohort with haematological malignancies than in those with solid tumours. Indwelling catheters, high-dose corticosteroids, high-risk haematological malignancies, metastatic solid tumours, high-intensity treatment, the presence of pneumonia, and tuberculosis increased the risk of sepsis and death in our patients. In HIV-infected patients with Non-Hodgkin's lymphoma, advanced disease, and severe immunosuppression due to the HIV-infection and chemotherapy, increased the risk of sepsis in this group of patients. The incidence risk ratio for pneumonia was 6.5, for tuberculosis 10.8, for invasive fungal infections 4.1, and for *Herpes* stomatitis 3.1 compared with the HIV-uninfected cohort.

<u>Conclusion</u>: Infectious complications following treatment in the Black South African child with cancer is a major burden, which contributes significantly to morbidity and mortality in this group of children. We need to pay greater attention to nutritional therapy (as the majority of patients were malnourished), aseptic techniques, monitoring of patients who are at risk for sepsis, and the judicious use of antibiotic therapy, paying heed to the principals of anti-microbial stewardship. Additionally there needs to be an increased vigilance for tuberculosis and pneumonia in children treated for cancer. Patients with haematological malignancies and HIV-infected children should be treated with Isoniazid prophylaxis for the course of the cancer therapy. Isolation facilities would help control the spread of infections in the unit. Increasing the awareness of childhood cancer will result in children being diagnosed with less advanced and aggressive cancer, which will ultimately decrease the infectious complications associated with intensive chemotherapy advanced surgery and extended-field radiotherapy for advanced disease. Early identification of HIV-infection, antiretroviral therapy and screening for malignant diseases in HIV-infected children may allow for earlier identification of AIDS-related malignancies.

ACKNOWLEDGEMENTS

- Dr Alane Izu, I am indebted to her for support, friendship and assistance with the statistics,
- Professor Shabir Madhi, my supervisor for his patience, encouragement, time, and guidance. I have learnt so much from him and am so grateful to have had him as my supervisor,
- The Cancer Association of South Africa for their funding,
- Dr Rosalind Wainwright, my mentor and teacher who has taught me Paediatric Oncology and without whose insistence I may not have completed this project,
- Dr Stelios Poyiadjis, who also taught me Paediatric Oncology and introduced me to the philosophical and spiritual aspects of treating children with cancer,
- Dr Diane McKinnon, with whom I have worked for many years and I have learnt so much from her including lateral thinking, rationality and compassion,
- Dr Biance Rowe, whose boundless enthusiasm, dedication and passion for our patients amazes me,
- Dr Mairi Bassingthwaithe, whose kindness, loyalty, and caring reinforces my belief in young doctors,
- The nursing, clerical, secretarial and cleaning staff in the Paediatric Haematology Oncology Unit, Chris Hani Baragwanath Academic Hospital without whom our unit would be non-functional,
- Dr Peter Adrian and Ms Gabisile Senna, who assisted with the collection and processing of the laboratory samples,
- Dr Jenny Vaughan, Dr Jeanette Wadula, Dr Pedro da Silva, Dr Paul Tsagane from the National Laboratory Health Services who assisted me with the technical details about laboratory testing,
- The patients and their parents, this study would not have been possible without you. I am humbled by your strength and optimism in the face of adversity,
- Ms Memory Chamwarura, for her help at home and the endless cups of tea,
- My siblings, Shireen, Komla and Mahesh for the memories we share,
- Hildegard Bell, my mother-in-law for her love, acceptance, kindness, and wisdom ,
- My parents Chilka and Seela, I am grateful for their love and nurturing, and for encouraging me to pursue a career in medicine. I owe so much to them,
- Christian, my husband, my friend, my strength, my support; I thank you for your love,
- and finally, Carl and Anil, my sons, to whom I owe so much happiness.

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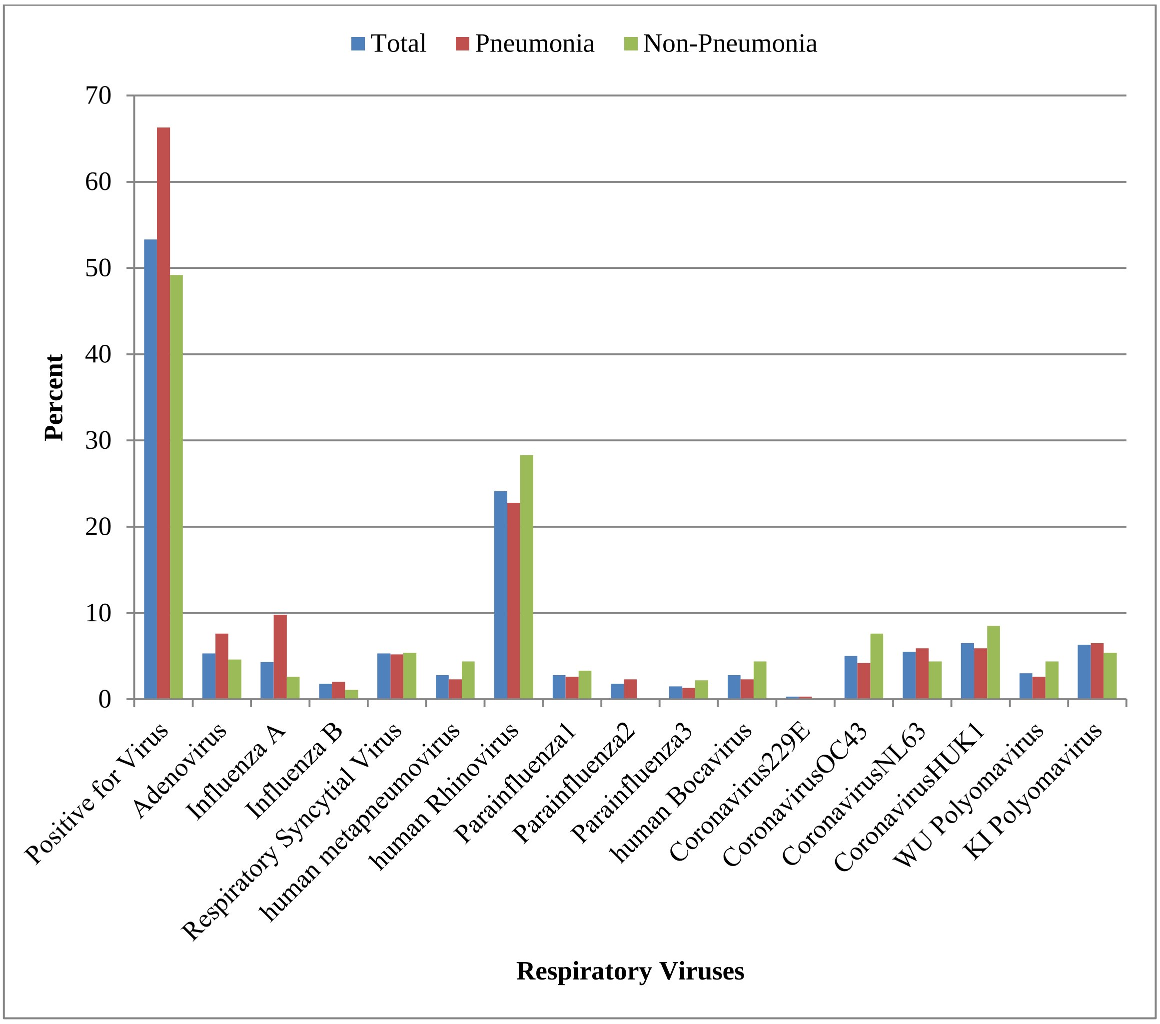
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Pneumonia
Non-Pneumonia
70
60
50
40
30
20
10
0
Percent
Positive for Virus
Adenovirus
Influenza A
Influenza B
Respiratory Syncytial Virus
human metapneumovirus
human Rhinovirus
Parainfluenza1
Parainfluenza2
Parainfluenza3
human Bocavirus
Coronavirus229E
CoronavirusOC43
CoronavirusNL63
CoronavirusHUK1
WU Polyomavirus
KI Polyomavirus
Respiratory Viruses

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ABBREVIATIONS

ACC	Adrenocortico-carcinoma
ADV	Adenovirus
ALC	Absolute Lymphocyte Count
ALL	Acute Lymphoblastic Leukaemia
AMA	Arm Muscle Area
AMC	Absolute Monocyte Count
AML	Acute Myeloid Leukaemia
ANC	Absolute Neutrophil Count
BMI	Body mass index
BSI	Blood stream infection
ARI	Acute respiratory infection
ARV	Anti-retroviral Therapy
BT	Brain Tumour
CABSI	Cather-associated blood stream infection
CFR	Case fatality ratio
CONS	Coagulase-negative *Staphylococcus*
CNSE	Culture-negative septic episode
CRP	C-Reactive Protein
ESBL	Extended-spectrum β-lactamase
FN	Febrile Neutropenia
G-CSF	Granulocyte colony stimulating factor

GCT	Germ cell tumour
GNB	Gram-Negative Bacteria
GPB	Gram-Positive Bacteria
HBL	Hepatoblastoma
HBoV	Human Bocavirus
HCoV	Human Coronavirus
HFA	Height-for-age
HIC	High-income Countries
HIV	Human Immunodeficiency Virus
HL	Hodgkin's lymphoma
HM	Haematological Malignancy
HMPV	Human Metapneumovirus
HPyV	Human Polyomavirus
HRV	Human Rhinovirus
ICU	Intensive Care Unit
IFI	Invasive fungal infection
IGRA	Interferon γ Release Assays
KIPyV	KI Polyomavirus
KS	Kaposi's sarcoma
LIC	Low-Income Countries
LMIC	Low- and Middle-Income Countries
LRT	Lower Respiratory Tract

LRTI	Lower Respiratory Tract Infection
MCSE	Microbiologically-confirmed septic episode
MIC	Middle-Income Countries
MRSA	Methicillin–resistant *Staphylococcus aureus*
MTB	*Mycobacterium tuberculosis*
MUAC	Mid-Upper Arm Circumference
NBL	Neuroblastoma
NHL	Non-Hodgkin's Lymphoma
NPA	Nasopharyngeal Aspirate
NPC	Nasopharyngeal Carcinoma
OS	Osteogenic sarcoma
PIV	Parainfluenza Virus
PPD	Purified protein derivative
RBL	Retinoblastoma
RMS	Rhabdomyosarcoma
RSV	Respiratory Syncytial Virus
RT-PCR	Real-time multiplex polymerase chain reaction
SD	Standard Deviation
SE	Septic Episode
SSE	Suspected Septic Episode
ST	Solid Tumour

TB	Tuberculosis
TST	Tuberculin Skin Test
TSFT	Triceps Skin Fold Thickness
URT	Upper Respiratory Tract
URTI	Upper Respiratory Tract Infection
VRE	Vancomycin-resistant *Enterococci*
WFA	Weight-for-age
WFH	Weight-for-height
WT	Wilm's Tumour
WUPyV	WU Polyoma Virus

1. INTRODUCTION

The survival of children with cancer in high-income countries (HIC) has improved with the use of aggressive chemotherapy, surgery, radiotherapy, and bone marrow transplantation (Pui et al., 2011, Winther et al., 2015). As a result of multi-modal therapy, these children are more susceptible to infections, which are a leading cause of death in this population (Adamski et al., 2008). Many host and disease factors increase the infectious complications in children undergoing treatment for cancer. Chemotherapy-induced bone marrow suppression, depress-ion of the immune system due to disease or treatment, mucosal barrier injury, intravascular devices, the type of cancer, chemotherapeutic proto-cols, surgery, radiotherapy and the duration of hospital stay have been recognized as risks factors for infections (Lehrnbecher et al., 1997).

In South Africa we have additional challenges of poverty, which contributes to low levels of parental education, unemployment, poor access to health care facilities, malnutrition, epi-demics of tuberculosis and HIV/AIDS and delayed presentation with advanced disease (Poyiadjis et al., 2011) which requires more aggressive treatment regimens.

A high incidence of sepsis has been demonstrated in paediatric patients receiving chemo-therapy; approximately 12.8% in children aged one to nine years and 17.4% in children aged ten to nineteen years, making febrile neutropenia (FN) a serious and worrying complication in paediatric cancer treatment (Yadav et al., 2014). In high-income countries (HIC), 16.4 per 1000 adult patients with cancer experience an episode of severe sepsis annually. Up to 8.5% of adult oncology deaths are due to severe sepsis (Williams et al., 2004b). There is a paucity of data regarding mortality and morbidity from infectious toxicity in children treated for cancer.

There are few studies on febrile illnesses in children from low-middle income countries (LMIC) (Gavidia et al., 2012), however, FN is a major cause of hospitalization in this patient group. In El Salvador, it has been documented, that treatment related complications were responsible for approximately 50% of deaths in children with acute lymphoblastic leukaemia (ALL) and acute myeloid leukaemia (AML) (Gupta et al., 2012b). Infections were the most common cause of treatment related mortality, with 12.3% of episodes of FN resulting in death (Gupta et al., 2011a). Similarly sepsis (53.3%) and bleeding (15.7%) were common causes of mortality in a cohort of Indian children treated for ALL (Yadav et al., 2011). Infectious complications in paediatric ALL makes it a particularly challenging disease to treat in LMIC (Gupta et al., 2009). Children who present with high-risk FN i.e. relapse of ALL, hypo-tension, an absolute neutrophil count (ANC) < 100 cells/µL, an absolute monocyte count (AMC) < 100 cells/µL, blood urea nitrogen ≥ 18 mg/dL, CRP ≥ 90 mg/L, and positive blood cultures, have a risk of death due to severe sepsis (Santolaya et al., 2007).

There is conflicting evidence about the susceptibility of children with solid tumours (ST) to chemotherapy-induced FN. Blood stream infections (BSI) and their aetiology in children with ST are under-reported.

The incidence of sepsis in oncology patients has increased globally over the past three decades, with up to 30% of deaths attributed to sepsis (Thirumala et al., 2010). This is perhaps due to the availability of intensive chemotherapeutic protocols, invasive procedures, immune-suppressive drugs, trans-plantation, and the increasing incidence of antimicrobial resistance. Severe underlying disorders are frequently (55% of 192 980) reported in patients with sepsis, with one in six having an underlying malignant disease (Angus et al., 2001) .

Health-care associated bacterial infections may be responsible for 25 000 deaths in African children annually, and its overall incidence in LMICs is double that in HICs (Allegranzi et al., 2011, Aiken et al., 2014). A recent South African study reported the crude infection mortality in children was 20%. High antimicrobial resistance rates, hospital-acquired sepsis, HIV infection, fungaemia, and Gram-negative bacteraemia significantly contributed to mortality (Dramowski et al., 2015).

A South African study investigated infections in children treated for cancer at Chris Hani Baragwanath Academic Hospital in 2001, prior to the onset of the HIV epidemic. This study documented a 14.3% incidence of bacteraemia (70% Gram-positive bacteria, 20% Gram-negative bacteria, and 10% fungal organisms). Fungi, Gram-negative bacteria (GNB) and Hickman lines were associated with a high mortality. Sixty-four percent of the fungal infections occurred in patients with Hickman lines, 42% of which were due to *Candida parapsilosis* (van de Wetering et al., 2001).

Since this study, there have been many changes in the Paediatric Oncology Unit at Chris Hani Baragwanath Academic Hospital. Patient numbers have more than doubled (Poyiadjis et al., 2011), Hickman lines were discontinued in favour of Porto-catheters, many treatment protocols for haematological malignancies (HM) and ST were intensified, the number of patients with HIV-related cancer has increased and there have been structural changes to the unit. The existing facilities were renovated and new ward and outpatient clinic were added on. The unit now has two in-patient wards and an outpatient clinic.

As fever and neutropenia frequently complicate paediatric oncology treatment and causes potentially life-threatening infections, the successful management of FN is based on careful monitoring and the prompt use of empiric broad-spectrum antimicrobials. It is imperative to document the pathogens isolated from patients with sepsis, their antimicrobial susceptibility patterns, and the clinical course of febrile neutropenia to optimize empiric treatment (Agyeman et al., 2014) .

The objective of the research reported in this thesis was to investigate the infectious related morbidity and mortality in predominantly black-African children treated for cancer at one of the largest hospital in the Southern Hemisphere, in Johannesburg, South Africa.

2. Review of the Literature

2.1. Survival In Children with Cancer

2.1.1. Childhood Cancer Survival in High-Income Countries

Between 1975 and 2009, the survival rates for all childhood cancers in the United States increased. The Surveillance, Epidemiology, and End Results programme, National Cancer Institute reported on survival data for paediatric childhood cancer for the periods between 1975 to 1979 and 2003 to 2009. Leukaemia survival improved from 48% to 84%, lymphoma from 72% to 91%, brain tumours (BT) from 59% to 75%, neuroblastoma (NBL) from 54% to 79%, retinoblastoma (RBL) from 92% to 99%, Wilm's tumour from 75% to 90%, bone tumours from 49% to 73%, and rhabdomyosarcoma (RMS) from 49% to 64% (Howlander, 2013). Some childhood cancers e.g. NHL and leukaemia are now considered curable. By contrast, the survival rates for children with certain ST, e.g. those with disseminated disease and most brain tumours have not improved significantly, with the exception of gonadal cancer, NBL and bone cancer (Pui et al., 2011). Studies from Europe confirm this improved survival in children with cancer (Lacour et al., 2014, Winther et al., 2015). However, the gains achieved by treatment intensification, individualised treatment and bone-marrow transplantation are lost by infection-related mortality and morbidity (O'Connor et al., 2014)

2.1.2. Childhood Cancer Survival in Low and Middle Income Countries

In 2010, 5.7 billion (83%) of the world's population of 6.9 billion people lived in LMICs. These countries have younger median ages and higher proportions of children in their populations than HICs. Overall, 27% of the population of middle-income countries (MIC) and 40% of the population of low-income countries (LIC) are younger than 15 years, compared with 17% of the population of HICs. GLOBOCAN estimated that about 148 000 cancers in children aged zero to fourteen years occurred in LMICs with a total population of 5.5 billion. In HICs, 28 000 cancers occurred in the same age group with a population of 1.2 billion (Ferlay et al., 2010).

The economies of most LMICs are characterised by poverty, too few health-care providers, weak health systems, poor access to education, and healthcare, and limited access to modern laboratory equipment. LMICs also have younger people, therefore have larger numbers of children with cancer than HICs (Magrath et al., 2013).

The cure-rates of cancer in children from HICs are significantly higher than in LMICs (Gupta et al., 2011a). This may be due to many factors, including delayed presentation with advanced disease, tumour biology and treatment toxicity in LMICs (Howard and Wilimas, 2005). Infection-related mortality contributes to overall survival in childhood cancer (Gupta et al., 2009).

Survival data of childhood cancer in LMICs is scanty. Data from India for the period 1990-2001, described the 5-year survival of 49.4% for Hodgkin's lymphoma, Wilm's tumour, RBL, NHL, Osteogenic sarcoma, ALL, and astrocytoma (Swaminathan et al., 2008). The overall survival rate for the period January 1995 to December 2004 in the Ivory Coast was 9.4% (Yao et al., 2013) and 54% in Zambia (Slone et al., 2014).

2.1.3. South African Childhood Cancer Survival

In South Africa, the overall survival for children with cancer for the period 1987 to 2011 was 52.1%. Lymphoma and WT had the highest survival rates at 63.9% and 62.6%, respectively. Brain tumours had the lowest survival rate at 46.4%. A comparison between ethnic groups showed white children had the highest survival (62.8%); 53.8% in children of mixed racial origin and 48.5% in black African children (Stones et al., 2014).

2.1.4. Racial and Socioeconomic Status and its Impact on Survival of Childhood Cancer

Despite advancements in the treatment of childhood leukaemia, socio-economic status may potentially affect disease prognosis. Children from low socio-economic sectors in the United States of America suffer two-fold higher death rates from ALL (Petridou et al., 2015). Treatment abandonment is a risk factor for poorer outcome in children treated for ALL. Rates of treatment abandonment is significantly lower in HICs than in LMICs (Gupta et al., 2013). Patients who live in the lowest socio-economic neighbourhoods at diagnosis had a 39% increased risk of death relative to those from higher socioeconomic neighbourhoods (Abrahao et al., 2015). Racial/ethnic differences in childhood cancer survival may be attributable to disease biology and host pharmacogenetics but are probably also linked to socioeconomic and cultural factors, including differences in access to health care, inadequate education, advanced disease stage at presentation, adherence to therapy and disbelief in modern medicine (Bhatia, 2011). However, some studies identified race/ ethnicity as an independent predicting factor (Viana et al., 2001, Bhatia, 2004).

There are also racial differences in the survival outcomes in children with ALL. Survival from ALL remains lower among non-White children in the US. Five-year survival is 85.0% for White, 81.4% for Asian, 79.0% for Hispanic and 74.4% for Black children (Abrahao et al., 2015).

2.2. The Spectrum of Infections in Children Treated for Cancer

2.2.1. Predisposition To Infections In Children Treated For Cancer

Patients who have cancer have a greater tendency to acquire infections than the general population. The critically ill cancer patient is at a high risk for infections and its resulting complications. Multiple factors are responsible for this heightened risk of infection. In addition to cancer treatments, mal-nutrition at diagnosis and during treatment, disruption of mucosal and integumentary barriers, neutropenia, cell-mediated and humoral immune dysfunction, presence of indwelling vascular catheters, local tumour effects, and prolonged duration of hospital stay contribute to the increased risk of infection. In this population, organisms with low virulence can cause significant morbidity and mortality. Bacteria, viruses, fungi, and protozoa can cause infection in the critically ill patient with cancer (Thirumala et al., 2010).

Various mechanisms are responsible for the risk of infections related to cancer treatment. Cortico-steroids, used for the treatment of ALL, NHL and BT, results in altered phagocytosis and defective cell-mediated immune responses which subsequently increases the susceptibility to fungal, viral and bacterial infections. Neutropenia, a major side effect of chemotherapy, contributes to immune-suppression and systemic bacterial and fungal infections. Methotrexate and purine analogues (the mainstay of treatment for ALL), decreases the number of T-cells and predisposes the individual to infections by *Pneumocystis jirovecii*, *Mycobacterium* species and viral infections. HM induces immunosuppression by the direct impairment of immune cells, including the number and functioning of T- and B-lymphocyte cells. Abnormalities of the innate and acquired immune system have also been recognized early in the course of ST (Lehrnbecher et al., 1997).

2.2.2. Bacterial Infections

Bacteria are the most common cause of microbiologically documented infections in the child with cancer (Hakim et al., 2010). Factors identified to increase the risk of invasive bacterial infection in children with febrile neutropenia (FN) include intensity of chemotherapy, stage of disease, mucositis, intravascular devices, depression of the immune function, prolonged neutropenia (> 7 days), profound neutropenia i.e. ANC < 100 cells/µL, immunosuppressive treatment, malnutrition, aggressive surgery, and wide-field radiotherapy. A study from the United Kingdom reported that infection-related mortality in children with cancer was 2.4%, accounting for 75 (64%) of 117 treatment related deaths. Sixty-eight percent of cases were associated with bacterial infection (64% GNB) and 20% with fungal infections (O'Connor et al., 2014). Studies from Italy, United States of America, and Germany have reported 50-79% of febrile illnesses in children are culture-negative) (Lehrnbecher et al., 2004, Castagnola et al., 2007, Hakim et al., 2010)

2.2.2.1. Bacterial Infections Complicating Childhood Cancer Treatment in Low and Middle Income Settings

Very few studies in LMICs have documented the incidence, microbiological aetiology, risk factors, and outcome of sepsis in children treated for cancer. In a prospective study from El Salvador, of 106 episodes of FN among 85 patients, 22% had a microbiologically confirmed sepsis and 12% of these resulted in death. Sixty-one percent of the bacteria were GPB, 48% GNB, including 48% being polymicrobial. Pneumonia was the only predictor of mortality (Gupta et al., 2011a). Infection is the commonest cause of treatment-related mortality in paediatric oncology patients treated in El Salvador (Gupta et al., 2012b). Studies from India have documented a predominance of Gram-negative bacteraemia (55.7%) in high-risk FN episodes (Ghosh et al., 2012). Gram-negative bacteraemia (46.7%) was twice as common as Gram-positive bacteraemia (23.3%) in children treated for ALL in India (Bakhshi et al., 2008). Data from Africa is limited, although it is proposed that sepsis-related mortality in African children treated for cancer may be higher than reported in HICs (Depasse et al., 2013, O'Connor et al., 2014).

2.2.2.2. Infections in Children with Solid Tumours

Kocak et al reported on a study to determine whether there were differences in patterns of infections and outcome of episodes of FN in paediatric patients with HM and ST. There was a diagnosis of fever of unknown origin in 73% and 74% of episodes in patients with HM and ST, respectively. Bacteraemia occurred in 18% with HM and 16% with ST. GPB were the most common in both groups. The median duration of fever was two days in both groups. The depth of neutropenia was similar, with 75% of HM and 70% of ST patients presenting with profound neutropenia. The median duration of neutropenia was nine days in patients with HM and six days in ST patients, while the median duration of antibiotic treatment was nine days and seven-and-a half days in the respective groups. There were no deaths in either group (Kocak et al., 2002).

2.2.2.3. Changing Epidemiology of Bacterial Isolates

Gram-negative bacteria were the dominant pathogens associated with FN in the 1960s, which was followed by the gradual emergence of GPB. This may have been due to the increased use of indwelling catheters and the evolution of new chemotherapeutic strategies (Aslan et al., 2012). In the last decade, Gram-negative pathogens in sepsis among cancer cases has increased, including the emergence of multi-drug resistant species (Menzo et al., 2015). In LMICs, Gram-negative bacteraemia may be more frequent, (Bothra et al., 2013) as the predominance of Gram-positive pathogens in HICs` is likely due to increased use of indwelling catheters, antimicrobial prophylaxis, and antacids which change the pH of the gastric contents .

Multi-drug-resistant Gram-negative bacterial species are causing an increasing number of infections in FN patients. *Klebsiella* species and *Escherichia coli* strains with acquired extended-spectrum β-lactamase (ESBL) genes frequently show a broad range of β-lactam antibiotic resistance. These ESBL producers are often only susceptible to Carbapenems, but the use of these antimicrobials may induce the selection of bacteria with broader resistance patterns. Infection with multi-drug resistant GNB is associated with increased morbidity and mortality (Trecarichi and Tumbarello, 2014). The majority of documented studies on the prevalence, risk factors, and outcomes of multi-drug resistant bacteria are from the adult population. In children with cancer, factors associated with multi-drug resistant Gram-negative bacteraemia are high-intensity chemotherapy and hospital-acquired bacteraemia (Haeusler et al., 2013).

2.2.3. RESPIRATORY VIRAL INFECTIONS IN CHILDREN

Approximately 150 million episodes of acute lower respiratory tract infection (LRTI) occur each year among children globally (Rudan et al., 2013). In children under two years of age, respiratory viruses cause most of these infections. Mixed viral-bacterial and virus-virus co-infections occur in 15-30% of cases (Nascimento-Carvalho et al., 2008), and in 25%-45% of patients, respiratory viruses can be detected in the absence of respiratory symptoms (Berkley et al., 2010). Bacterial co-infection with respiratory viruses increases morbidity and mortality (Dawood et al., 2014), but the impact and clinical implications of co-infection with multiple respiratory viruses is unclear.

The incidence of paediatric acute LRTI is highest in LMICs. In sub-Saharan Africa alone, more than 35 million episodes occur each year, resulting in 500 000 deaths (Rudan et al., 2013). More than 90% of acute LRTI deaths occur in LICs (Black, 2003, Williams et al., 2004a).

Real-time multiplex polymerase chain reaction (RT-PCR) is automated, less laborious, and less expensive than conventional methods. Hence, it may be more suitable for LMICs (Jartti et al., 2013). Recent studies using RT-PCR postulated a substantial role for respiratory viruses in paediatric LRTI episodes in LMICs (Berkley et al., 2010, Pretorius et al., 2012). An infection from respiratory viruses in healthy children is often self-limited; however, such infections can cause significant morbidity and mortality in the immunocompromised children.

2.2.3.1. Respiratory Viral Infections in HIV-Infected Children

Immunocompromised children, irrespective of the cause of immunodeficiency, are at increased risk to develop viral infections, have more severe disease and a poorer outcome than immunocompetent children. In a study carried out in Soweto, South Africa, Madhi et al showed that HIV-infected children are at increased risk of respiratory virus associated severe LRTI (Madhi et al., 2000a). In another study, Madhi et al also found that HIV-infected children are more likely to develop RSV and influenza-associated pneumonia and have a greater fatality ratio than HIV-uninfected children (Madhi et al., 2004). Another South African study (8723 children < 5 years of age, hospitalized with LRTI, 12% HIV-infected and 64% less than 12 months of age) reported the prevalence of respiratory virus identification to be 78%, including 37% for human Rhinovirus (HRV), 26% for Respiratory Syncytial Virus (RSV), 7% for influenza virus and 5% for human Metapneumovirus (HPMV) (Cohen et al., 2015).

2.2.3.2. Respiratory Viral Infections in Paediatric Oncology Patients

Bacterial and fungal infections have been the focus of research in cancer patients, as infections caused by these pathogens are treatable. Recent advances in viral diagnostics have produced sensitive and accurate methods for detecting viruses, with quick results and these tests are able to screen for multiple species and subtypes (Debiaggi et al., 2012, Jartti et al., 2013).

In the USA and Europe, in children with cancer, respiratory viruses were detected in 40-75% of paediatric oncology patients presenting with febrile illnesses, making respiratory viruses an important cause of febrile illnesses in such children (Christensen et al., 2005, Koskenvuo et al., 2008, Lindblom et al., 2010, Torres et al., 2012b, Srinivasan et al., 2013, Benites et al., 2014). This may have the potential to individualise infection treatment, and to tailor the extensive use of antibiotics in immune-compromised children with FN. These studies, however, did not include a control group. Hence, the significance of identification of some of the respiratory viruses is uncertain as they have also been identified in asymptomatic healthy children, (e.g. HRV, human Bocavirus) and need further investigation in immunocompetent and immunocompromised children.

In both HICs and LMICs, respiratory diseases are responsible for a high proportion of morbidity and mortality in childhood. It is estimated that 25% to 33% of deaths in children one to 59 months of age are caused by acute respiratory infections (ARI) and their complications. Respiratory infections are also the most common syndrome associated with febrile illnesses in children < 5 years age, including in children treated with anti-neoplastic drugs (Koskenvuo et al., 2008). Many protocols are designed for the management of FN in children with cancer. However, there are still doubts regarding the true incidence and the role of respiratory viruses in ARIs in these patients (Christensen et al., 2005, Koskenvuo et al., 2008, El Saleeby et al., 2008b). In addition to only a few studies being published on this subject, there is also a paucity of data on more recently discovered viruses such as human Coronaviruses (HCoV), human Polyomaviruses (HPyV), human Bocavirus (HBoV) and HMPV in immunocompromised paediatric patients (Gerna et al., 2006). Furthermore, there is a paucity of data on the role of multiple respiratory viruses in children with cancer, which could increase the risk of severe illness (Nair et al., 2010).

Respiratory virus associated infections have been documented in children undergoing treatment for HM and ST (Christensen et al., 2005). This may explain the poor responses to antibiotics in certain children, and the high proportion of febrile episodes for which aetiological agents are not identified on blood culture in children with cancer, and are deemed fever of unknown origin. However, certain fungi e.g. *Aspergillus* are not identified on blood culture.

Severe RSV infection is more likely in children with cancer who are receiving immunosuppressive chemotherapy. The greater the severity of immunodeficiency, the higher the risk is of RSV infection, of progression to LRTI and of RSV-related mortality (Chemaly et al., 2014). HRV was the most commonly identified virus in paediatric patients with cancer and acute LRTI and fever (Hirsch et al., 2013) and in adult patients (Ohrmalm et al., 2012). The significance of identifying HRV in the pathogenesis of an illness is however unclear, since HRV is also frequently identified from the healthy population (Jacobs et al., 2013) In paediatric leukaemia and HSCT patients, symptomatic para-influenza virus infections have been reported to range from 2 to 7%, one-third of which manifest as LRTI (Chemaly et al., 2012). HMPV was detected in 55 immunocompromised children in Seattle, USA, between 2006 and 2010, 44% of whom had a HM, 16% were undergoing haemopoietic stem cell transplantation , and 16% had a ST. Twenty-three percent of these required intensive care support (ICU) support, including 5% with HMPV-associated pneumonia (Chu et al., 2014). Disseminated HBoV infection in the immunocompromised patients has also been described in a few reports, although in some cases co-pathogens are also present and the clinical relevance of HBoV detection is unclear (Campbell et al., 2015).

Haematological malignancies are considered a major risk factor for severe influenza virus infection (O'Riordan et al., 2010). Prolonged duration of clinical symptoms, a high rate of concurrent bacteraemia and considerable chemotherapy delay has been reported (Feldman et al., 1977). These early studies remain concordant with findings 30 years later (Tasian et al., 2008). The clinical patterns of HPyV infection has not been clearly described in immunocompetent or immunodeficient groups of patients (Debiaggi et al., 2012) . Infections with WUPyV and KIPyV in children with cancer occurred in older children (Mourez et al., 2009). Severe adenovirus (ADV) infections have been reported in immunocompromised patients, such as transplant patients, those with inherited and acquired immunodeficiency states, and patients undergoing chemotherapy (Steiner et al., 2008).

More than one virus has been detected in some FN episodes in children treated for cancer (Koskenvuo et al., 2008, Suryadevara et al., 2012b, Srinivasan et al., 2013, Benites et al., 2014). HRV is the most common respiratory virus detected, either as a sole virus or in combination with other viruses (Suryadevara et al., 2012a, Hirsch et al., 2013, Srinivasan et al., 2013, Benites et al., 2014). There are conflicting opinions on the significance of the presence of HRV in children with a respiratory illness. Children with viral infections complicating FN have a more benign illness and favourable outcome if a single virus was the only positive finding for a febrile infection (Torres et al., 2012a, Suryadevara et al., 2012b, Srinivasan et al., 2013). Another study described fatal viral associated infections in children with cancer (Mendoza Sanchez et al., 2006).

Two studies have evaluated the association of chemotherapy intensity on the epidemiology of respiratory virus infections in children treated for AML and ALL. The frequency and severity of ARI was increased in children treated with more intensive chemotherapy regimens, with fatal outcomes from respiratory viruses, 14% in children treated for AML (Sung et al., 2009) and 31% during the induction phase therapy in ALL cases (Salzer et al., 2009).

The degree of bone marrow suppression is associated with respiratory viral infections in children treated for cancer. Prolonged and profound neutropenia and lymphopenia increase the predisposition to respiratory viral infections in children with cancer (Koskenvuo et al., 2008, Srinivasan et al., 2013, Choi et al., 2013). Some studies have only documented an association with neutropenia and respiratory virus infections in children treated for cancer (Benites et al., 2014) and others have only documented lymphopenia as a risk factor for respiratory virus infections (El Saleeby et al., 2008b).

An important consequence of respiratory virus infections is the delay in the chemotherapy protocol. Chemotherapy protocols, especially in the paediatric population are designed in a metronomic fashion with multi-modal drug combinations and delays in the schedule may lead to drug resistance and relapse of disease with adverse outcomes. Forty to 50% of oncology patients experienced a median delay of 7 days in scheduled chemotherapy due to respiratory virus infections (Mendoza Sanchez et al., 2006).

2.2.3.3. RESPIRATORY VIRAL AND BACTERIAL CO-INFECTION IN CHILDREN WITH CANCER

Many microorganisms, both commensals and pathogens, colonize the upper respiratory tract. Respiratory viral infections can increase the bacterial load by changing the physical barriers and the immune system.

Studies have detected a high bacterial load in approximately 40% of lower respiratory tract samples from paediatric RSV infections (Hishiki et al., 2011), which can lead to secondary complications such as pneumonia (Madhi et al., 2004, Madhi et al., 2006). Usually the bacteria co-detected with respiratory viruses are opportunistic pathogens, such as *Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae,* and *Moraxella catarrhalis* (Honkinen et al., 2012).

Viral-bacterial co-infections are a common and clinically significant problem. An immune-compromised state is a risk factor for mortality in children with a variety of respiratory viral pathogens, including RSV, influenza virus, and ADV (Spaeder et al., 2013). Patients with bacterial-viral co-infections often have more severe disease and delayed responses to antibiotics than those with a sole bacterial infection (Koskenvuo et al., 2007) or sole respiratory virus infection (Torres et al., 2012b). The role of respiratory viruses as sole infecting pathogens or as co-infections with bacteria and or fungi as a cause of overall FN episodes in children with cancer has not been well investigated and characterized compared to bacterial infections in children with cancer.

Co-infections with bacteria or fungi complicate respiratory virus infections in paediatric oncology patients (El-Mahallawy et al., 2005, El Saleeby et al., 2008b). Fifteen percent of paediatric oncology cases with influenza virus had simultaneously diagnosed bacteraemia. Concomitant pathogens included *Pseudomonas aeruginosa*, *Enterobacter cloacae, Enterococcus faecalis*, and Coagulase-negative *Staphylococcus* (CONS) (Tasian et al., 2008).

Patients with bacterial-viral co-infections had more severe illnesses and slower response to antibiotics than those with a sole bacterial infection (Koskenvuo et al., 2007).

The increased susceptibility to bacterial co-infection in individuals with respiratory viral infections has been reported for RSV and influenza virus. This includes many of the fatalities of the 1918–1919 influenza pandemic which were caused by secondary bacterial pneumonia (Klugman and Madhi, 2007). Respiratory viral infections assist bacterial adhesion by altering physical and immune system barriers. Respiratory viruses increase the ability of bacteria to infect or adhere to mucosal surfaces by alterations in the host cell membranes. Respiratory viruses can also damage the lung epithelia thereby increasing bacterial trans-location. Exudates on mucosal surfaces resulting from respiratory virus infections may also increase bacterial growth. The neuraminidase protein from the influenza virus plays an active role in thinning the mucus and exposing receptors on epithelial cells, leading to increased bacterial translocation and infectivity (Peltola et al., 2005). The host immune defence against bacteria can be affected by respiratory virus infections through the inhibition of nonspecific phagocytosis, or by exacerbating the effect of bacterial toxins. Influenza virus infection can inhibit neutrophilia, which allows an increase in bacterial infection (Bordon et al., 2013). Respiratory viruses may also increase the expression of host receptors used by bacteria to enter cells, particularly platelet-activating receptor, a key factor for *Streptococcus pneumoniae* infection (Grigg, 2012). Bacterial co-infection occurs during the dampening of the immune response. Interleukin-10 is the cytokine involved in the resolution of the immune response, but it can lead to an increase in bacterial infections (van der Sluijs et al., 2004). A general down regulation of pathogen sensing may also occur following a respiratory virus infection, leading to an increased incidence of bacterial infection (Tregoning and Schwarze, 2010, Chorazy et al., 2013).

Examples of co-infecting bacterial and viral pathogens are common in the literature, but reports tend to be virus-specific. For example, most deaths associated with influenza pandemics are associated with secondary bacterial infection including *Streptococcus pneumonia* and *Haemophilus influenzae*.

The importance of viral and bacterial co-operation in cases of pneumonia is growing (Honkinen et al., 2012). Pavia et al. conducted a study among patients hospitalized with community-acquired pneumonia and demonstrated that HRV-pneumococcal co-infection was independently associated with severe pneumonia (Pavia, 2013). Johannson et al. demonstrated that HRV-bacterial or HCoV-bacterial co-infections were independently associated with severe pneumonia (Johansson et al., 2010). Another study of children with community-acquired pneumonia suggested that mixed viral-bacterial co-detections were associated with treatment failure (Honkinen et al., 2012).

Respiratory virus infections in children receiving anticancer chemotherapy were also investigated for in a paediatric oncology unit in the Western Cape, South Africa. Evidence of a viral and bacterial infection was found in 31 (30%) and 24 (24%) episodes, respectively. Within these, a combined viral and bacterial infection was demonstrated in six (6%) episodes. Thirty-five viral isolates were identified in 31 febrile episodes. Infectious agents were more likely to be of bacterial (30%) than viral (15.5%) origin (Bouw et al., 2007).

2.2.4. Fungal Infections

Fungi are an important cause of infections in patients with prolonged neutropenia. Neutropenic patients who remain febrile despite a course of broad-spectrum antimicrobial therapy are prone to fungal disease. *Candida* and *Aspergillus* species. are the most common fungi responsible for invasive fungal infections (IFI) in children. They are associated with a high mortality and morbidity rate. In infants and children, invasive candidiasis is five times more frequent than invasive *Aspergillus*. *Candida* species represents the third most common agent found in healthcare-associated BSI in children. Invasive aspergillosis is more often associated with HM than ST. *Candida albicans* is the main *Candida* species associated with invasive candidiasis in children (Brissaud et al., 2012).

The incidence of IFIs has increased in paediatric cancer patients over the last decade, and is a major cause of morbidity and mortality. This may be due to the use of aggressive cancer protocols, aggressive surgery, and radiotherapy with its consequent immunosuppression and increase in the number of bacterial infections and the subsequent use of broad spectrum antibacterial agents (Castagnola et al., 2011). Prolonged and profound neutropenia (Nesher and Rolston, 2014), severe mucositis, the use of broad-spectrum antibiotics and high-dose corticosteroids, and invasive procedures contribute to this increase in the incidence of IFIs in the child with cancer (Groll et al., 2014). In the child with febrile neutropenia, *Candida* and *Aspergillosis* are the most common fungal pathogens causing IFIs (Pappas et al., 2009, Segal et al., 2011). Invasive candidiasis has a case fatality ratio approximating 10% (Zaoutis, 2010) while *Aspergillosis* has a fatality ratio between 30% and 50% (Steinbach, 2010). HM, especially AML and chemotherapy- induced bone marrow suppression is a well-recognized risk factor for IFIs (Villarroel et al., 2010).

The identification of fungal pathogens is extremely challenging in the paediatric population. Fungal culture from a sterile site remains the gold standard of identification but fungal cultures lack sensitivity. Invasive procedures for histological diagnosis may be life threatening in the child with neutropenic sepsis, thrombocytopenia, and septic shock. Imaging studies lack specificity (Roberts et al., 2012). Galactomannan antigen detection has a low sensitivity in children (Zou et al., 2012) and ß D-glucan studies have many false positive results in the child previously treated with cell-wall inhibitor antibiotics, as it the first-line empiric antibiotics in febrile children with cancer (Lamoth et al., 2012). In LMICs, imaging studies may not be easily and timeously available, galactomannan testing is not routinely available, and ß D–glucan studies have a slow turn-around time, which limits the usefulness of these tests for the diagnosis of IFIs.

2.2.5. Polymicrobial and Mixed Bloodstream Infections in Patients with Cancer

Polymicrobial infections are responsible for approximately 15% of infections in immune-compromised patients with cancer. There is limited information regarding the spectrum and microbiology of these infections, even in severely neutropenic cancer patients (Rolston et al., 2007). Polymicrobial infections in children with cancer are poorly documented (Hung et al., 2003). Blood stream infections (BSI) are a growing worldwide concern because of their potential for severe consequences. Most BSIs are monomicrobial, but according to the largest series reported, polymicrobial BSIs account for 6%–34% of BSIs (Sutter et al., 2008). Polymicrobial BSIs are usually associated with a higher morbidity and more severe prognosis than monomicrobial BSIs. Microorganisms causing polymicrobial BSIs are usually combinations of GPB and GNB or *Candida* species. However, infections with mixed bacterial and *Candida* species is a neglected entity of uncertain clinical significance (Bouza et al., 2013).

2.2.6. Pneumonia in Children with Cancer

Pneumonia is a common clinical syndrome in children with cancer and a strong predictor of mortality (Ghosh et al., 2012, Bakhshi et al., 2008). Bacterial pathogens are the most common cause of ARIs complicating cancer chemotherapy. *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae,* and *Enterococcus faecalis* are the most common GPB, whereas *Escherischia coli, Pseudomonas aeruginosa,* and *Klebsiella* species are the most common GNB associated with pneumonia in children with cancer. *Mycobacterium tuberculosis* (MTB) is also a cause of pneumonia in the immunocompromised patient. Factors associated with high mortality are bacteraemia, refractory neutropenia, and a delay in appropriate antibiotic treatment. Despite appropriate treatment, more than 50% of patients die of progressive infection or haemorrhage (Thirumala et al., 2010). Common respiratory viruses causing acute respiratory tract infections include influenza, parainfluenza virus (PIV), RSV, cytomegalovirus, and herpes simplex virus. RSV accounts for 30% to 49% of all respiratory viruses in patients who are immunocompromised and have HM. In cancer patients, RSV usually presents as an upper respiratory tract infection (URTI) that can progress to fatal pneumonia (Shachor-Meyouhas et al., 2013). Patients who have prolonged and profound myelosuppression, and lymphopenia, and exposure to high-dose corticosteroids, are at the highest risk for progressing from pneumonia to death. The single most important predictor of mortality in influenza-associated pneumonia is absolute lymphopenia. Invasive pulmonary aspergillosis can occur in patients who are neutropenic and is commonly fatal with a mortality rate as high as 60%. *Pneumocystis jirovecii* pneumonia (PCP) is less common among patients who have cancer compared with patients who have HIV infection. The incidence among patients with HM is higher than those with ST. Corticosteroids are a major risk factor for PCP. Other predisposing factors include intensity of chemotherapy and a low CD4+ lymphocyte count.

Acute respiratory failure often requires admission to the ICU in patients with cancer and is associated with high mortality. In recent years, several studies have reported an increase in survival rates in cancer patients requiring mechanical ventilation for acute respiratory failure. Investigators have attributed these higher survival rates to recent advances in oncology and critical care management of these patients.

2.2.7. Tuberculosis

Young children and immunocompromised children of all ages, are at high risk of acquiring tuberculosis (TB) and progressing to infection and of developing more severe forms of the disease including disseminated disease (Zar and Pai, 2011). It is estimated that children make up 10% to 15% of the global burden of TB (Marais et al., 2006b). The relationship between latent TB and active TB depends on the balance between the immune system and *Mycobacterium tuberculosis*. A latent state or active TB will ensue, depending on the balance between the microbe and the immune system. Various individual or environmental factors favour the development of active disease such as HIV status or immunodeficient conditions (Zumla et al., 2013).

2.2.7.1. HIV Disease and Tuberculosis in Children

Tuberculosis rates are at extremely high levels in countries with a high burden of HIV infection. TB remains one of the most frequent causes of morbidity and the primary cause of death in HIV-infected adults in sub-Saharan Africa, with southern Africa at the epicentre of the dual epidemic. The high TB incidence rates among HIV-infected children may be explained by an increased *Mycobacterium tuberculosis* exposure and or an increased vulnerability to develop active TB following exposure (Marais et al., 2011).

Immunocompromised children are a highly susceptible group for TB infection. HIV-infected infants have a 20-fold increase in TB incidence compared to HIV-uninfected infants. The risk of TB is four times higher in children with CD4+ percentage < 15% than among those with CD4+ percentage > 15% (Marais et al., 2011).

2.2.7.2. Cancer and Tuberculosis

Since 1970, the American Thoracic Society and Centre for Disease Control and Prevention have recognized that cancer increases the risk of TB especially in those patients with ALL and HL (Cliffton and Irani, 1970). The incidence of TB is high in patients with HM because of defects in the T-cell component of the immune system, which is caused by both the underlying disease and its treatment. The relative risk of TB disease in adult patients with HM is two to 40 times that of the general population. Neutropenia, impaired humoral and cellular immunity are frequent complications in patients with HM. Neutrophils produce a number of mycobactericidal cytokines and chemokines. In adults with cancer, the risk of latent TB infection has been shown to be inversely proportional to the baseline ANC (Martineau et al., 2007).

It is unclear about the timing of TB reactivation in patients with HM. Re-activation could result from impaired immunity caused by the underlying malignant process or by the chemotherapy-induced immunosuppression or by re-infection. Progression to active TB in immunocompromised individuals might contribute to the high burden of TB in cancer patients. The risk of developing TB can vary depending on the type of cancer. In adult patients, chronic lymphocytic leukaemia (Silva et al., 2005) and AML (Chen et al., 2011) have been reported as risk factors for re-activation of latent TB. There are underlying immunological deficiencies which facilitate the emergence of infections in patients with HM (Pagano et al., 2012). Alteration in the Th1 cell response of the HM itself or high doses of corticosteroids leads to an impaired immune response, which may promote the progression from latent TB to active TB (Al-Anazi et al., 2007).

There are reports of TB in children undergoing chemotherapy from both high burden (Karakas et al., 2003) and low burden countries (Lancioni et al., 2009, Klossek et al., 2004). In a study from South Africa, the incidence of TB in children treated for cancer was 9117 per 100 000 years, which was 22 fold greater than the incidence in the general population of children (Stefan et al., 2008b).

2.2.7.2.1. The Diagnosis of Tuberculosis

It is imperative to diagnose TB in patients with cancer and to ensure early treatment is initiated to eliminate the *Mycobacterium tuberculosis* before a deterioration in the immune system increases the risk of active TB (Anibarro and Pena, 2014). TB is difficult to diagnose in children because the clinical presentation and radiological signs may be non-specific. Young children rarely present with cavitatory lung disease, hence TB in children is often pauci-bacillary. The diagnostic gold standard for active TB relies on the detection of TB by culture methods. Sputum-smear microscopy is positive in < 10-15% of children with TB, and culture yields are low (30-40%), but may be higher in children with advanced disease (Marais et al., 2006c) Diagnosis is even more difficult in children with HIV-infection with its resultant malnutrition and anergy, further impeding the interpretation of the TST tests (Zar and Pai, 2011). Additionally, acute pneumonia is a common disease in children from LMIC which adds to the challenges of diagnosing TB (Singh and Aneja, 2011).

At present, tests used to diagnose latent TB include the tuberculin skin test (TST) and two interferon γ release assays (IGRA) assays (Quantiferon- TB Gold-in-Tube (QFT) and T-SPOT.TB (T-SPOT)(Pai et al., 2014). The IGRA tests are based on the detection of interferon-γ released from sensitized lymphocytes against specific antigens of TB.

2.2.7.2.2. The Tuberculin Skin Test

The TST is a standard method used to determine whether a person is infected with MTB. The TST measures the delayed type of hypersensitivity response to the purified protein derivative (PPD) also known as tuberculin. The Mantoux technique is the preferred method of PPD administration. The test is performed by injecting 0.1 ml of PPD intradermally on the volar aspect of the forearm. The skin test is read between 48 and 72 hours after administration. The reaction is measured in millimetres of induration i.e. the hard, palpable, and raised area of swelling. The diameter of the induration is measured perpendicular to the long axis of the forearm. A positive Mantoux response is defined as ≥ ten mm (≥ five mm in an HIV-infected child or severely malnourished child). A positive TST does not indicate disease, but, infection with MTB.

A negative TST does not exclude TB infection or disease, and may be due to disseminated (miliary) TB and/or TB meningitis, HIV-infection (or other viral infections such as measles), immune-suppressive drugs e.g. corticosteroid therapy, severe malnutrition and recent TB exposure (2-3 month delay in conversion) (Moore 2009).

2.2.7.2.3. The T-SPOT.TB Test

The killing and clearance of invading pathogens are mediated by T-effector cells. These cells have a short life and die-off when the pathogen is cleared. Hence, their continuing presence indicates the cellular immune response is currently encountering a pathogen somewhere in the body. Measurement of effector T-cells in a sample therefore diagnoses an on-going infection.

This is the basis of the T-SPOT.TB test. The T-SPOT.TB® technology is designed to detect and quantify effector T cells (Lalvani et al., 2001).

T-cells release interferon γ together with a number of other cytokines, which detects and destroys infecting organisms. T-cells recognize specific epitopes on the infecting organism, which initiates the release of cytokines. The T-SPOT.TB test uses very specific antigens (ESAT-6 and CFP 10) to stimulate the effector T-cells.

The Centres for Disease Control recommend the use of IGRA tests in children, but there is little evidence to support this (Mazurek et al., 2010). In the immunocompromised, very young, HIV-infected or malnourished child, IGRA may have a role in diagnosis. However, as with the TST, a positive test does not equate with active TB disease (Zar and Pai, 2011). IGRA sensitivity, like with the TST test, is diminished by HIV infection and low CD4+ lymphocyte counts have been associated with higher rates of indeterminate IGRA results (Lalvani and Pareek, 2015).

A study in adult patients with cancer concluded that neither IGRA nor TST can conclusively rule out active TB in the immunocompromised patient (Jung et al., 2012). The diagnosis of TB in children with cancer is particularly challenging. A South African study assessed the use of the TST and two IGRA tests for the detection of MTB infection in children with cancer before initiating chemotherapy treatment. TST and IGRA results were discordant, with fewer positive results than expected. The TST and IGRA tests performed sub-optimally in this group of children, and therefore none of them could be used in isolation to exclude MTB infection (Stefan et al., 2010) The sensitivity of the tests for detecting latent TB infection in patients with underlying immunodeficiency is lower than that in patients with conserved immunity (Redelman-Sidi and Sepkowitz, 2013). Very few studies have evaluated the value of IGRAs in patients with cancer. Piana et al compared TST and T-SPOT.TB test in 138 adult Italian patients with HM who had been in contact with a smear-positive TB patient. Of these patients, 17% were positive for the TST compared to 44% who were positive to the T-SPOT.TB test (Piana et al., 2006). Richeldi et al found a similar result in 95 Italian patients with HM. The percentage of positive results for IGRAs was higher than that for the TST (26% for QFT, 18% for T-SPOT.TB compared to 10% for the TST). However, the authors cautioned that IGRA does not have greater sensitivity than the TST for detecting latent TB infection in this group of patients (Richeldi et al., 2009).

A recent study by Moon SM et al compared the TST and QFT in 244 South Korean patients before HSCT. Most of the patients were vaccinated with BCG. The percentage of positive results was not significantly different (10% for the TST with a cut-off of 10 mm against 16% for the QFT) (Moon et al., 2013).

Immunosuppressed patients have a higher prevalence of indeterminate results with both the T-SPOT.TB test and QFT. In a recent report of 18 children with cancer or primary immunodeficiencies who had been exposed to an adult with smear-positive TB, the T-SPOT.TB test had fewer indeterminate results than the QFT (Carvalho et al., 2013). In another study with 34 South African children with cancer, indeterminate results were found in four patients (11%) with the T-SPOT.TB technique, as against 15% when using the QFT. Indeterminate results were more frequent in children with HM than in those affected by other types of malignancy (Stefan et al., 2010).

2.2.8. MALNUTRITION

It is estimated that approximately 826 million people in the world are undernourished. In 2013, fifty-one million under-five year olds were wasted and 17 million were severely wasted. Approximately two thirds of these children lived in Asia and almost one third in Africa (Unicef, 2015).

In 2008, 8.8 million children under the age of five years died globally, 93% occurred in Africa and Asia. Malnutrition is directly or indirectly responsible for 54% of deaths in children under five, and contributes to every second death (53%) associated with infectious diseases among children under five years in these countries (WHO, 2013).

The HIV pandemic contributes to childhood malnutrition. De Maayer et al documented clinical outcomes of severe malnutrition in a high TB and HIV setting in Johannesburg, South Africa. Fifty-one percent of 113 severely malnourished children were HIV-infected. The overall case death rate was 11.5%. HIV-infected children were six times more likely to die than HIV-uninfected children (De Maayer and Saloojee, 2011).

2.2.8.1. The Interaction between Malnutrition and Infection

Malnutrition is the most common cause of immunodeficiency worldwide and increases the vulnerability of the host to infections (Enwonwu et al., 2006).The immune system first executes the innate and then the acquired host defence functions, in response to infections. Both processes consume anabolic energy, and the mediators of inflammation increase the catabolic response. Hence, the nutritional status of the host determines the outcome of the infective process (Schaible and Kaufmann, 2007).

Protein energy malnutrition causes atrophy of lymphoid tissues, particularly T-lymphocyte areas, and causes the thymus to involute, which results in the arrest of lymphocyte development, reduced numbers of circulating mature CD4+ helper cells and impairment of antibody production to T-dependent antigens. The lymphocytes are reduced in number and activity (Beisel, 1996). Malnutrition also reduces complement formation, which results in a decrease in phagocytosis of infecting pathogens. Patients with malnutrition have phagocytes with reduced bactericidal and candidicidal effects (Rytter et al., 2014). The gut mucosa is atrophied and permeable in malnourished children (Prendergast and Kelly, 2012).Cell-mediated immunity is impaired in malnourished children, which in turn increases the susceptibility to intracellular pathogens, reactivation of viral infections and susceptibility to developing opportunistic infections (Schaible and Kaufmann, 2007).

2.2.8.2. Childhood Cancer and Malnutrition

Children with cancer are vulnerable to malnutrition, because they have increased substrate needs due to the disease and its treatment (Bauer et al., 2011). At the same time, children have increased requirements of nutrients to achieve appropriate growth and neurodevelopment (Han-Markey, 2000). Malnutrition in children with cancer is mainly due to a decreased intake and reduced absorption of nutrients caused by gastrointestinal side effects of cancer therapy, primary effects of the cancer itself, and changes in metabolism. Malnutrition contributes to impaired immune function, delayed wound healing and altered drug metabolism which impacts negatively on the disease outcome (Tisdale, 1997). A decreased tolerance, altered metabolism of chemotherapy and an increase in infection rates in malnourished individuals has been described (Gomez-Almaguer et al., 1998, Zimmermann et al., 2013). Underweight children are less likely to complete their treatment and are at high risk for relapses and mortality as compared to normally grown children (Khan et al., 2006).

Body mass index (BMI) and changes in BMI during treatment may influence treatment outcomes of paediatric patients with ALL. Being underweight at diagnosis is a risk factor for relapse, and a decrease in BMI early during treatment is associated with decreased survival (den Hoed et al., 2015). Information regarding the clinical relevance of weight loss during treatment for childhood cancer is lacking, but in adults, weight loss >5 % or a decrease ≥ 1.0 in BMI Z-score, in the first three months after diagnosis was considered relevant (Brinksma et al., 2012).

Malnutrition is a significant prognostic factor for survival of children with cancer, especially those with ST and metastatic disease (Rickard et al 1986), NBL (Viana et al 1994), ALL and AML (Lobato-Mendizabal et al 2003) and RMS (Burke et al., 2013). The Children's Oncology Group investigated the effect of malnutrition on event-free survival and found that only those children with sustained malnutrition were at significantly greater risk for relapse or death and development of treatment related mortality (Orgel et al., 2014).

A study set in Malawi to investigate the relationship between malnutrition and neutropenia in children treated for Burkitt's lymphoma demonstrated that malnutrition in children at diagnosis was associated with increased chemotherapy-induced profound neutropenia. Acute malnutrition at diagnosis as assessed by arm muscle area (AMA) was associated with a higher rate of neutropenia, prolonged neutropenia, febrile neutropenia, delays in treatment and death during treatment. Four (4.9%) of the Burkitt's lymphoma cases died of treatment related causes, including three due to Gram-negative septicaemia. All patients with profound neutropenia (12, 14.8%), prolonged neutropenia (7, 8.6%), and treatment related deaths (4, 4.9%) were malnourished at diagnosis (Israels et al., 2009).

2.2.8.3. Risk Factors for Malnutrition in Children with Cancer

Malnutrition is associated with the type, stage, and metastatic status of the tumour (Rickard et al 1996). Patients who present at diagnosis with malnutrition often have advanced disease and or unfavourable biology (Barron et al 2007). Lack of family and health care support may lead to nutritional deprivation (Bauer et al 2011). Dysphagia, pain, early satiety, anorexia, nausea, vomiting, diarrhoea, oral and intestinal mucositis, anal fissures and alterations in smell and taste (van Bokhorst-de van der Schueren et al 2005) contribute to malnutrition in children with cancer.

2.2.8.4. Malnutrition in children with cancer in Low Middle Income Countries

Eighty five percent of the world's children live in LMICs. Infectious diseases, malnutrition, lack of health care facilities (Sala et al., 2004, Sala et al., 2005) and late presentation with advanced disease adversely affect these children. The prevalence of malnutrition in children diagnosed with cancer in developing countries is 50% (Sala et al., 2004). Malnutrition also correlates with low socio-economic status (Viana et al 2001). Malnutrition, poor housing conditions, low income, difficulties with communication and transportation, lack of health and laboratory services, contribute to a greater risk of recurrence in children with ALL (Lobato-Mendizabal et al 1991).

Malnutrition at diagnosis is positively associated with treatment abandonment, one of the major reasons for a decreased survival outcome, in the developing world (Sala et al., 2012, Antillon et al., 2013). Malnutrition and low socio-economic status results in advanced disease at diagnosis, variability in drug metabolism (Murry et al 1998) and a high rate of abandonment of treatment (Barr et al 2000).

The prevalence of malnutrition in children diagnosed with WT in South Africa was recorded at 31% (Wessels et al., 1999). In Malawi, children with cancer and underlying acute malnutrition at diagnosis, had a higher rate of neutropenia, prolonged neutropenia, profound neutropenia, febrile neutropenia, delays in treatment and death during treatment than those who were not malnourished (Israels et al., 2008). In a study from Guatemala, more than 50% of the patients had some degree of malnutrition at diagnosis, reflecting both a possible delay in diagnosis and the general condition of the population (Sala et al., 2008). Malnutrition was documented in 52% of children with ALL (kumar et al., 2000) and 56.8% of all cancers (Jain et al., 2003) in newly diagnosed cancer cases among Indian children. In Bangladesh, 53% of children are malnourished at diagnosis. These children had two to three times more microbiologically documented and clinically proven infections, required a longer duration of induction and prolonged hospital stay (Hafiz and Mannan, 2008). In the children with severe mal-nutrition, after 6 months of treatment, the hazard of death was 2.4 fold the hazard of those who were well nourished or moderately malnourished (Sala et al., 2012, Antillon et al., 2013). Malnourished children more often abandoned therapy and their event free survival was inferior to that of other children (Sala et al., 2012).

2.2.8.5. Diagnosis of Malnutrition

Body composition is assessed is using weight and height or length, from which the BMI (weight/height ^{2}) is calculated. WHO Anthro and WHO Anthro-plus calculators calculate weight-for-height (WFH), weight-for-age (WFA), height-for-age (HFA), and their Z-scores. BMI-for-age defines underweight as < 15th centile and severe malnutrition as < 5th centile. The classification of the World Health Organization defines malnutrition as WFA ≤ -2SD;

stunting as HFA ≤ -2SD and wasting as WFA ≤ -2SD (de Onis and Blossner, 2003) . A loss in body weight of ≥ 5% constitutes acute mal-nutrition and a HFA value < 5th percentile may reflect chronic undernourishment in children (Smith et al., 1991).

Weight may be an inaccurate measure in children with large tumour volumes, which may weigh more than 10% of their body weight (Sala et al., 2004). Measurement of nutritional status at diagnosis of childhood cancer relies almost exclusively on weight-related indices. Studies have compared the use of arm anthropometry with weight. There is strong evidence that malnutrition is common at the time of diagnosis in children with cancer, and arm anthropometry should replace the use of weight-related indices to identify malnutrition in children (Oguz et al., 1999). Mid-upper arm circumference (MUAC) provides an estimate of fat-free mass, which is very similar to lean body mass, while triceps skinfold thickness (TSFT) yields an estimate of fat mass (Barr et al., 2011, Webber et al., 2013).

There are different ways of evaluating nutritional status. Arm anthropometry is independent of tumour mass in children (Oguz et al., 1999). The most essential information in evaluating nutritional status is the lean body mass. MUAC and TSFT are also useful, both of which are used to determine the AMA. The lean body mass is calculated by measuring the MUAC and TSFT (i.e. muscle + fat) and triceps skin fold (i.e. fat only). AMA is then calculated from the MUAC and the TSFT, using the following equation: AMA = $(\text{MUAC mm} - \pi\text{TSFT mm})^2/4(\text{MUAC mm})$ (Frisancho, 1981).

Biochemical markers such as plasma protein, pre-albumin, retinol-binding protein, albumin, and transferrin are not particularly useful in children with cancer and in critically ill children. Infection, fever, and fluid shifts change these variables, as they are acute phase reactants (Brennan et al 1998).

2.3. IMMUNOLOGY

Children with cancer have disruptions in the host defence which interfere with the responses to infections and preventing establishment of long-term immunity (Lehrnbecher et al., 1997).

Cancer patients display varying degrees of immunosuppression at the time of presentation, prior to initiation of therapy. This has been described with ALL and other conditions associated with pancytopenia at the time of presentation. Prolonged and intensive chemotherapy administered with or without corticosteroids and radiotherapy, adds to the immunosuppression. Patients with HL have impaired lymphocyte proliferation to a variety of antigens, and patients with Burkitt’s lymphoma have variable levels of lymphocyte depletion, which relate to the stage of disease. Even patients with ST e.g. sarcomas, a disease not normally associated with immunosuppression, occasionally show reduced peripheral blood T-cell populations at the time of presentation (Mackall, 2000).

Chemotherapy affects leukemic cells and normal T- and B- cells, and reduces immunoglobulin levels. Immune abnormalities may persist for months after the end of therapy. The degree of immune suppression may be related to the intensity of treatment and the time to complete recovery may be longer for the T- than for the B-cell compartment (Eyrich et al., 2009).

2.3.1. INTEGUMENTARY BARRIERS

The systemic effects of the chemotherapy and the local effects of radiation on the oral mucosa cause mucositis. Chemotherapy and local radiotherapy damage the oral and gastrointestinal mucosa. Pro-inflammatory cytokines are released; these cause increased mucosal permeability and translocation of commensal bacteria and fungi, which colonizes the mucosal surfaces. This results in localized or systemic infection. Thereafter, cell repair and tissue healing occur (Bow, 2013).

Younger patients are at higher risk of chemotherapy-induced oral mucositis, because their epithelium has a higher mitotic rate. The most common complication of mucositis, especially with neutropenia, is an increased predisposition to bacteraemia, septicaemia, and fungaemia. *Streptococcus mitis* and *Streptococcus oralis* are the most frequently isolated bacteria. Mucositis can also be the starting point for fungal infection, by *Candida albicans*, as well as other types of *Candida* species and *Aspergillus* (Sonis, 2004, Villa and Sonis, 2015).

The skin and mucosal surfaces are the primary host defence and protects against invasion by endogenous and acquired microorganisms. Specialized cells of the skin, respiratory tract, gastrointestinal tract and genitourinary tract protect the body from invading microbial agents (Lehrnbecher et al., 1997). The integrity of the integumentary barrier may be disrupted by invasion of local tumour, radiotherapy, surgery, and or chemotherapy. Many procedures, for example finger prick testing, venepuncture, bone marrow aspiration and insertion of venous catheters, can also disrupt the integument and provide a nidus for colonization and dissemination of pathogens (Dhainaut et al., 2005).

2.3.2. Neutrophils

Neutrophils are the first cells recruited to the site of an infection and contribute to the acute inflammatory response. Studies in adults cancer patients showed that serious bacterial infections occur with an absolute neutrophil count of < 500 cells/µL (Bodey et al., 1966). This was later confirmed in paediatric studies (Pizzo et al., 1982). Neutropenia may be secondary to the underlying cancer e.g. ALL or a result of chemotherapy or radiotherapy. Studies have shown that neutrophils from patients with leukaemia have sub-optimal chemo-attractive responsiveness, bactericidal activity, and superoxide production. In patients with AML, neutrophils have abnormal adhesiveness, migration, phagocytic function, and killing activity of *Staphylococcus aureus* and *Candida albicans*. Chemotherapy and radiotherapy also alter neutrophil function resulting in the depression of superoxide regeneration, phagocytosis and depressed microbicidal activity (Lehrnbecher et al., 1997).

The incidence of infections in a neutropenic patient is 11 to 38 % (Lehrnbecher et al., 1997, Akova et al., 2005). The depth and duration of neutropenia is important. The likelihood of developing a microbiologically documented bacterial infection is almost 100% when a patient remains neutropenic for three or more weeks without antibiotic treatment (Dhainaut et al., 2005).

Severe neutropenia is defined by an ANC of < 500 cells/µL and profound neutropenia by an ANC < 100 cells/µL. The incidence of infection was reported to be 14% if the neutrophil count fell below 500-1000 cells/µL and 24-60% if the ANC fell to < 100 cells/µL. The longer the duration of neutro-penia, and the more rapid the of decline in white cell count, the greater the incidence of infection. If the granulocytopenia was prolonged for > five weeks, then the incidence of infection was 100% (Lehrnbecher et al., 1997). Individuals who remain neutropenic develop secondary fungal or recurrent bacterial infections frequently associated with reduced antibiotic susceptibility. These infections may be refractory to standard therapy and are associated with a poorer prognosis (Bodey et al., 1966, Donowitz et al., 2001, Akova et al., 2005, Sepkowitz, 2005).

The median time to the first neutropenic fever corresponding to the time of the neutrophil nadir occurs at the end of the second week, typically between days ten and 14, from the first day of cytotoxic therapy. This is related to the time of the maximum cytotoxic effect of chemotherapeutic agents on the intestinal mucosa and the time of maximal oral and gastrointestinal mucositis. Neutropenia is an indirect marker for mucositis (Pizzo, 1993).

The rate of decrease circulating neutrophils is also important, as patients with a rapidly decreasing ANC after therapy for ALL are at greater risk for infectious complications than are patients with chronic neutropenia such as aplastic anaemia (Koh, 2012).

2.3.2.1. Granulocyte Colony Stimulating Factors

Granulocyte colony stimulating factors (G-CSF) is administered immediately after a course of myelotoxic chemotherapy to decrease the depth and duration of neutropenia and the associated risk of infection. Early trials documented that the use of G-CSF in patients receiving myelotoxic chemotherapy can reduce the incidence of febrile neutropenia. Patients who received G-CSF had a shorter duration of neutropenia, faster recovery from fever and a shorter duration of antibiotic use (Mhaskar et al., 2014). The American Society of Oncology guidelines recommend the use of G-CSF for primary prophylaxis if the expected rate of fever and neutropenia is greater than 40% (Bennett et al., 1996).

2.3.3. Lymphocytes

Red blood cells, leukocytes, and platelets are short-lived, and are replaced continuously. Post-chemotherapy, these cells are replaced within 14-21 days. T-cells are comprised of short and long-lived cells, which are not capable of rapid mitotic division; hence, the restoration of T-cells after chemotherapy is a slow process.

Therapy-induced T-cell immunosuppression predisposes the patient to potentially fatal opportunistic complications. Patients with ALL are predisposed to infections with *Pneumocystis jirovecii* and other opportunistic infections. Leukaemias originate and spread in the primary and secondary lymphoid organs, therefore, these patients present with depleted lymphoid populations. Chemotherapy protocols are intensive and prolonged, which further adds to the depletion of lymphocytes. Cytotoxic chemotherapy and radiotherapy for ALL (MacLennan and Kay, 1978) and ST (Yovino et al., 2013) depletes T-cells, with CD4+ cells being more affected than CD8+ cells. Opportunistic infections occur in patients with CD4+ counts < 200 cells/µL. The abnormalities in T-cell function, which are seen post chemotherapy, are due to a combination of T-cell depletion and intrinsic functional defects (Lehrnbecher et al., 1997, Mackall, 2000).

Transient lymphopenia is a risk factor for nosocomial infections. Profound lymphopenia is an independent predictor of RSV-related LRTI in children with cancer (El Saleeby et al., 2008a). Prolonged lymphopenia is also a risk factor for nosocomial sepsis, multi-organ failure and death (Gurevich et al., 1995).Abnormalities of lymphocytes affect both the humoral and cellular components of the immune system. Patients with defects in the humoral system are susceptible to infections by encapsulated bacteria such as *Streptococcus pneumoniae, Haemophilus influenzae,* and *Neisseria meningitides.* Patients with NHL and HL have defects in their cellular immunity.

Cycle length of chemotherapy is determined by the time necessary to achieve safe values for neutrophils and platelets. Adequate time for lymphocyte reconstitution may not occur and could result in clinical complications related to immune incompetence. Mackall et al. studied ten children and young adults who were treated for cancer. The time between cycles was adequate for granulocytic, monocytic and platelet recovery but lymphocytes did not recover before the administration of successive cycles, which led to severe B-cell and T-cell depletion in these patients and all developed severe opportunistic infections (Mackall et al., 1994).

2.3.4. GLUCOCORTICOIDS

Glucocorticoids are an integral component of the treatment for HM and brain tumours. Gluco-corticoids increase the risk of infections by inducing functional changes in cellular metabolism, vascular permeability and wound healing. They depress phagocytic function, suppress intracellular microbicidal activity, inhibit granulocyte adherence, block granulocyte aggregation and suppress the release of chemotactic factors which results in a decrease in the accumulation of phagocytes at the site of inflammation (Lehrnbecher et al., 1997). Corticosteroid exposure is an important risk factor for infection outcomes in children treated for cancer and, more specifically, is independently associated with both sepsis and infection-related mortality (Dix et al., 2012) .

2.4. INDWELLING CATHETERS

Long-term venous access devices have facilitated the administration of chemotherapy antibiotics, blood products, fluids, feeds and collection of blood samples and these have improved the quality of life for patients with cancer. However, the use of indwelling catheters can lead to bloodstream infections, referred to as catheter-associated bloodstream infection (CABSI) (Tomlinson et al., 2011).

CABSI infections are related to the extra-luminal colonisation of the catheter, which originates from the skin, and from haematogenous seeding of the catheter tip, or intraluminal colonisation of the hub and lumen of the central venous catheter. Haematogenous seeding with organisms originating from the gastrointestinal tract is seen in patients who are neutropenic and those with mucositis.

Patients with cancer with indwelling catheters are at particular risk for CABSI. Signs and symptoms of these infections may be altered in patients with cancer due to neutropenia or steroid administration (Boersma et al., 2008). CABSI in neutropenic patients may be unaccompanied by inflammation at the catheter site. Central venous catheter-related infections are common, 0.5–2.8 per 1000 catheter days in children. They are associated with increased mortality, increased length of hospital stay, treatment interruptions, and increased complications in adults (Chesshyre et al., 2015) and in children with cancer (Celebi et al., 2013).

Coagulase-negative *Staphylococcus aureus*, GNB, and *Candida albicans* are most common infections associated with indwelling catheters. Non-MTB infections are also associated with indwelling catheters (Marschall et al., 2014). Septic thrombi and infective endocarditis are life-threatening complications of indwelling catheters. Prolonged neutropenia contributes to the number of CABSI infections.

2.5. Markers Of Sepsis

Despite modern algorithms for resuscitation and new antimicrobials, sepsis-related morbidity and mortality remain high. Bacterial infections are life threatening for neutropenic patients; therefore, early diagnosis of these severe infections is imperative for management of patients with sepsis. Infections account for 70% of fatal complications in ALL (Crokaert, 2000). Careful clinical examination with attention to vital signs, a high index of suspicion and knowledge of the local bacterial isolates, including bacterial sensitivity are vital to early diagnosis and management, especially in LMIC settings. In neutropenic patients, bacteraemia can occur without major clinical findings, except for a high fever, but can rapidly lead to septic shock with multi-organ failure and death (Laws, Schneider et al 2000). Children who present with high-risk FN i.e. relapse of ALL, hypotension,, ANC < 100 cells/µL, AMC <.100 cells/µL, blood urea nitrogen ≥ 18 mg/dL, CRP ≥ 90 mg/L, and positive cultures, have a risk of death due to severe sepsis (Santolaya et al., 2007).

The most precise way to diagnose bacterial infections is by culture but there are often delays in results. The identification of early markers of bacterial infections could guide treatments, reduce the misuse of antibiotics and possibly improve long-term outcomes of patients treated for sepsis (Velicer et al., 2004). In HICs, C-reactive protein (CRP) and procalcitonin (PCT) have been investigated in both the adult and paediatric populations among immunocompetent and immunocompromised individuals, as markers of sepsis. However, few, such studies have evaluated their diagnostic and prognostic significance in the African setting.

In patients with cancer, many non-infective factors may mimic severe infection. These are the administration of drugs and blood products, a high-cell turnover e.g. T-cell ALL and Stage 4 Burkitt’s lymphoma, and spontaneous tumour lysis syndrome may lead to systemic inflammation and mimic severe infection.

The relationship between malnutrition and acute phase reactants remains uncertain. Some authors maintain that CRP production, in response to infections, is unaffected in the patient with malnutrition (Malave et al., 1998). Children with kwashiorkor have reduced rates of protein breakdown and synthesis, but their CRP levels were similar to those without kwashiorkor (Manary et al., 1998). On the other hand, Page et al reported that CRP and PCT were not sufficiently accurate for diagnosing invasive BSIs in patients with severe acute malnutrition (Page et al., 2014).

2.5.1. C-Reactive Protein

Interleukin 6, a sepsis-related cytokine, stimulates CRP production by the liver (Verboon-Maciolek et al., 2006, Hengartner et al., 2004). CRP then binds to polysaccharides in pathogens, which activates the classical complement pathway. Plasma concentrations are elevated in patients with bacterial and fungal infections (Du Clos et al 2000). CRP has a low specificity therefore is not a very reliable marker for sepsis (Petrola, Toikka et al 2007), has limited power to discriminate between infectious and sterile inflammations (Schuttrumpf et al., 2006) and has a prolonged time to reach peak levels. CRP secretion starts within four to six hours after stimulation, peaking only after 36 hours and has a half-life of six hours (Gendrel D, Raymond J et al 1999).

High levels of CRP at admission were predictors of sepsis in a Chilean cohort of children with cancer (Santolaya et al., 2008). A Greek study documented that CRP levels were slightly lower in patients with non-bacterial infections, but CRP was elevated in all conditions related with febrile neutropenia, including non-bacterial episodes (Hatzistilianou et al., 2010).

2.5.2. Procalcitonin

Procalcitonin was described as a new marker of infection in 1993 (Gendrel et al., 1999). PCT production is induced in all tissues by bacterial infection (Muller et al., 2001). Under normal conditions, negligible serum PCT is detected but can reach up to levels of 1000 ng/ml in severe infections (Bernard, Ferriere et al 1998). The concentration changes of the PCT molecule is stable and easy to determine, making it a useful marker (Hatzistilianou et al., 2002).

PCT is produced by the liver and peripheral blood mononuclear cells in response to infection. PCT rises in response to bacterial or fungal infections is related to the severity of the infection. PCT levels rise within four hours, peak at six hours and then plateau at eight to 24 hours after endotoxin injection (Dandona 1994). PCT has an elimination half-life of approximately 25 to 30 hours (Meissner et al., 2000). In non-neutropenic patients, PCT secretion increases three to eight hours from the beginning of the infection with a plateau at twelve to 24 hours (Tang et al., 2007).

In the paediatric population, several publications reported on the role of high PCT levels in systemic bacterial and parasitic infections (Simon et al., 2008). PCT is thought to be able to discriminate between bacterial and respiratory viral infections (Gendrel et al., 1999). In both neutropenic adult and paediatric patients, high levels of PCT have been documented in bacterial and fungal infections (Ruokonen et al., 1999). PCT serum concentration also has been demonstrated to better predict the presence of bacterial infection compared to CRP (Simon et al., 2008). In children with febrile neutropenia, PCT was more sensitive and specific than CRP for the diagnosis of Gram-negative bacteraemia (Fleischhack et al., 2000).

In a review of the literature, PCT was discriminative in distinguishing fever due to systemic forms of infection from non- infectious aetiologies. Patients with IFIs may have a delayed increase in PCT levels. PCT has a minimal role, if any, in discriminating Gram-negative from Gram-positive infections. and is not superior to CRP (Sakr et al., 2008).

PCT concentrations are lower in neutropenic than in non-neutropenic patients with bacterial infections (Hatzistilianou et al., 2010). Possible reasons for this include that neutrophils are one of the sources of PCT production (Hitoglou et al., 2001) and that liver cells which also produce PCT may be damaged by chemotherapy (Christ-Crain et al., 2004).

2.6. HIV Lymphomas

2.6.1. The Epidemiology of HIV Lymphomas

Despite medical, social, and political interventions, the number of people living with HIV continues to increase. In 2014, the number of people newly infected with HIV was 2 million (220 000 in children < 15 years) and, 1.2 million (150 000 in children < 15 years) deaths were due to AIDS (WHO, 2013).

Approximately five to ten percent of HIV-infected individuals develop NHL, which is an AIDS-defining illness in approximately three percent of HIV-infected individuals (Remick, 1995). The risk of developing NHL increases with the duration of HIV-infection and the depth of immunosuppression. Increased survival is due to the introduction of anti-retroviral therapy (ARV), but long-term survivors of HIV-infection may remain at increased risk for NHL (Engels et al., 2008).

The incidence of NHL in sub-Saharan Africa did not increase at the rate it did in the North American HIV-infected population and this was attributed to mortality from infectious complications in LMIC, which prevented the development of malignancies (Parkin et al., 1999). It may also be due to under-reporting as the most common clinical diagnosis of persistent lymphadenopathy is TB and not NHL (Naresh et al., 2011).

Individuals diagnosed with AIDS during childhood remain at an elevated risk for malignancy into adult-hood, despite treatment with ARVs. HIV-associated lymphomas often present with a more aggressive histology and advanced stage. Impaired bone marrow reserve and underlying immune-deficiency contribute to higher rates of infectious complications compared with immunocompetent patients with NHLs (Mounier et al., 2007). The baseline CD4+ lymphocyte count at the time of NHL diagnosis was significantly associated with subsequent death in adults, being strongest for those with baseline CD4+ counts < 50 cells/µL (Barta et al., 2013).

In Africa, overall survival of NHL in HIV-infected children is poor. The median survival in Uganda was 11.8 months (Orem et al., 2009). In another study, the overall survival at 2 years from diagnosis was 59% for HIV-uninfected NHL and 30% for HIV-infected patients with NHL (Chao et al., 2010). In South Africa, the overall disease-free survival for all HIV-infected children with cancer is 33.7%., 10.2% died of treatment toxicity including infectious complications (Davidson et al., 2014). Early in the HIV epidemic, treatment of HIV-infected patients diagnosed with NHL was mainly palliative, with median survival measured in months (Straus et al., 1998). The introduction of ARVs in 1996 resulted in reduced morbidity and mortality from HIV-infection, thus allowing more aggressive NHL therapy(Levine, 2008).

2.6.2. Drug Interactions between Antiretroviral Therapy and Chemotherapy

Many chemotherapeutic drugs and ARVs are metabolized in the liver through the cytochrome p 450 enzyme system. ARVs can increase or decrease the clearance of chemotherapeutic drugs by alterations of the cytochrome p 450 system, resulting in increased chemotherapy-associated toxicity or a decrease in the efficacy of ARVs (Floyd et al., 2006).

Many of the chemotherapeutic drugs and ARVs have similar toxicities. Zidovudine causes myelo-suppression in 8% of individuals. Didanosine and Stavudine cause peripheral neuropathy, which can add to the same effect caused by the Vinca alkaloids. HIV protease inhibitors cause nausea and vomiting which is the most common adverse effect of chemotherapy. Nucleoside analogues cause nephrotoxicity; protease inhibitors and non-nucleoside reverse transcriptase inhibitors cause liver toxicity. Ritonavir is an inhibitor of Vinca alkaloids and alkylating agents. CD4+ counts decline by > 50% in patients treated with chemotherapy and ARVs (Powles et al., 2002). The combination of ARV therapy and chemotherapy augment each other's toxicities and add to the complexities of treatment of children with NHL and HIV disease.

2.6.3. BACTERIAL INFECTIONS IN HIV-INFECTED CHILDREN

A progressive impairment of the cell-mediated and humoral immunity is seen in patients with HIV-infection. T-cell defects predispose to many opportunistic infections, and HIV causes B-cell dysfunctions, which lead to impaired humoral responses and increased risk of bacterial infections. Children with advanced HIV disease cannot mount an adequate humoral immune response to bacterial pathogens, especially encapsulated bacteria. HIV-infected children have increased rates of sino-pulmonary and other serious bacterial infections commonly caused by *Streptococcus pneumoniae* and other bacteria. Bacterial infections are a major source of morbidity and mortality in HIV-infected children (Jaspan et al., 2008). HIV-infected children have a greater risk of bacterial infections than their HIV-uninfected counterparts, and these infections are more aggressive and invasive, and more likely to have poorer outcomes. In addition, infections in HIV-infected children are complicated by polymicrobial infections (McNally et al., 2007).

Pneumonia, urinary tract infection, cellulitis, abscess, chronic otitis media, and skin infections are more common in HIV-infected individuals. *Streptococcus pneumoniae, Salmonella* species, *Haemophilus influenzae* type b, and non-typhoid *Salmonella* are the most frequently cultured bacteria in HIV-infected individuals. The mortality of bacteraemia in HIV-infected patients is between seven to 46% (Huson et al., 2014). Pneumonia is the commonest cause of death in HIV-infected children, both with and without cotrimoxazole prophylaxis.

Multi-drug resistant bacteria are a global problem, in both HIV-infected and HIV-uninfected children. The resistance of both *Streptococcus pneumoniae* and *Staphylococcus aureus* to cotrimoxazole is significantly higher in the HIV-infected group's isolates in Soweto (Madhi et al., 2000a). There is a high burden of bacterial resistance in both Gram-positive and Gram-negative bacterial isolates from HIV-infected children (Madhi et al., 2000b, Cotton et al., 2008, Jaspan et al., 2008, Groome et al., 2012).

2.6.4. Pneumonia in Children with HIV

Pulmonary complications occur in up to 70% of HIV-infected patients , mainly of infectious aetiology (Benito et al., 2012). HIV-infected children with pneumonia have advanced disease, bacteraemia, infections with *Pneumocystis jirovecii*, high rates of treatment failure, and death compared with HIV-uninfected children (Gray and Zar, 2010). Bacteria are the leading cause of severe pneumonia in HIV-infected children. *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Escherichia coli*, and *Salmonella* species are the leading bacteria causing infections. The incidence of bacterial pneumonia is indirectly proportional to the decline in CD4+ count. Eighty percent of cases of bacterial pneumonia occur with a CD4+ count < 400 cells/µL and recurrent pneumonia with a CD4+ counts < 300 cells/µL (Hirschtick et al., 1995).

Pneumocystis jirovecii is a frequent cause of pulmonary infections in HIV-infected children hospitalized with severe pneumonia (Morrow et al., 2014). *Pneumocystis jirovecii* and other fungal pneumonias often develop in patients whose CD4+ count is < 200 cells/µL (Lee et al., 2015). At least one third of HIV-infected persons are infected with MTB, and HIV-infection is the largest risk factor for developing TB (Swaminathan et al., 2010). Patients with advanced immunosuppression present with advanced TB and extra-pulmonary disease (Martinson et al., 2011). TB is the commonest cause of morbidity and the leading cause of death in HIV-infected adults in sub-Saharan Africa (Corbett et al., 2006).

There have been many changes in the Paediatric Oncology Unit at Chris Hani Baragwanath Academic Hospital since the 1990s. The number of children being treated for cancer has more than doubled, Porto-catheters have replaced Hickman lines, many treatment protocols for HM and ST were intensified, autologous stem cell transplants are routine treatment, the number of HIV-infected patients has increased, and there have been structural changes to the Oncology unit, including a new in-patient and outpatient treatment facility. Furthermore, as is the case globally, the antibiotic sensitivity pattern has likely changed over the last two decades in

South Africa, although this has not been recently documented in our paediatric oncology setting.

The aim of this research program reported in this thesis was to investigate the infectious related morbidity and mortality in predominantly black-African children treated for cancer at one of the largest hospital in the Southern Hemisphere (Johannesburg, South Africa) and in a setting with a high prevalence of HIV-infection.

3. METHODOLOGY

3.2. AIM OF THE STUDY

The overall aim of the study was to prospectively delineate the epidemiology of infectious morbidity and mortality in African child with cancer. Additionally, we aimed to evaluate the usefulness of indirect markers and risk factors for sepsis in these children.

3.3. OBJECTIVES OF THE STUDY

1. To investigate the burden of bacterial sepsis in HIV-infected and HIV-uninfected children treated for cancer (Chapter 4);
2. To investigate the role of respiratory viral infections as possible etiologic agents in sepsis cases among children treated for cancer (Chapter 6);
3. To compare the tuberculin skin test to the quantiferon γ release assay (T- SPOT.TB) for diagnosing *Mycobacterium tuberculosis* infection in children with cancer on chemotherapy (Chapter 5);
4. To evaluate the role of nutritional status in predisposing to sepsis in African children with cancer (Chapters 4, 5, 6);
5. To compare the infectious disease morbidity in HIV-infected and HIV-uninfected children treated for NHL with the same chemotherapy protocol (Chapter 7);
6. To evaluate the usefulness of C-reactive protein and procalcitonin as markers of bacterial sepsis in children with cancer (Chapters 4, 5, 6)

3.4. SETTING OF THE STUDY

The study was carried out in the Paediatric Oncology Unit at Chris Hani Baragwanath Academic Hospital (CHBAH). The hospital has a wide referral area, including Soweto, southern Gauteng, Mpumalanga, and North West province. It is a secondary-tertiary level care hospital and a teaching hospital for the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

The Paediatric Oncology unit consists of two in-patient wards, one for children age 0 to 7 years, and the other for those aged 8-19 years. The unit also has an outpatient clinic and a residence facility in Soweto, where children who do not need to be hospitalised can be housed with a parent. The home-facility offers accommodation, meals, and transport to the hospital for emergencies and treatment.

3.5. Study Design

This prospective single centre study enrolled all patients from (one year of age to 19) admitted with cancer to the Paediatric Oncology Unit, Chris Hani Baragwanath Academic Hospital from April 2009 to March 2011. The children were followed-up until the completion of their treatment or up until the end April 2014, when the study ended. The events studied were all febrile episodes in children who received treatment (including chemotherapy, irradiation, and or surgery) for HM or ST. The standard care for these patients included a detailed history and physical examination, blood investigations (full blood count with a differential and a blood culture for a suspected septic episode). In addition, patients enrolled into the study had anthropometric measures undertaken on admission (Appendix 2), a TST by the Mantoux method and blood was drawn to undertake the T-SPOT.TB test. For each septic episode, a detailed history (Appendix 3) was taken, a standardized physical examination performed (Appendix 4), blood was obtained for culture, full blood count, CRP and PCT (Appendix 5), a nasopharyngeal aspirate was performed (Appendix 6), and empiric antibiotic treatment was initiated (Appendix 7), which was tailored, if necessary, based on the blood, urine or other culture results.

3.6. ETHICS

All individuals older than eight years were assented and their parents or legal guardians provided written informed consent for participation in this study (Appendices 8, 9, 10). Ethics clearance was obtained (Appendix 14) from the Human Research Ethics Committee, University of the Witwatersrand (Ethics clearance number: M080304).

3.7. STATISTICAL METHODS

3.7.1. BINARY OUTCOMES

For binary outcomes, proportion and percentages were reported. The chi-square tests or Fisher's exact tests were used to determine if significant differences existed between two proportions for un-clustered data. For clustered data, a generalized mixed effect logistic regression model was used to compare a single explanatory variable with a dependent binary outcome. Multiple logistic regression were used to calculate an adjusted odds-ratio, which adjusted for the effects of confounding factors that were statistically significant (p-value $\leq$ 0.05) from the simple regression analysis.

3.7.2. QUANTITATIVE OUTCOMES

The mean and standard deviation was reported for variables assumed to be normally distributed. Students t-tests were used to determine effects on normally distributed outcomes for un-clustered data. For clustered data, a linear mixed effects regression model was used to determine significant association between an explanatory variable with a normally distributed outcome. Log-transformations were used when appropriate.

For non-normal data, medians were reported and the Wilcoxon rank sum test (for un-clustered data or clustered data) was used to determine significant associations.

For count data, the incidence and 95% Confidence Intervals (95% CI) were reported. Follow-up time was calculated as the number of months from date of presentation of disease to the last follow-up visit, which was either loss to follow-up, refusal of further treatment, end of treatment or death. A Poisson regression or mixed effect Poisson regression was used to determine significant association for un-clustered and clustered data, respectively. A p-value ≤ 0.05 was considered statistically significant.

3.8. Study Definitions

3.8.1. Fever

Fever was defined as a temperature of ≥ 38.5°C as a single reading or ≥ 38 °C twice within one hour (Link et al., 2003, Freifeld et al., 2011).

3.8.2. Bacteraemia and Fungaemia

Bacteraemia or fungaemia was defined as bacteria or fungi cultured from blood under sterile conditions. If CONS was cultured on a single blood sample, it was presumed to be a contaminant. However, if cultured twice from blood within 48 hours it was considered a significant pathogen (Link et al., 2003, Freifeld et al., 2011). We only report on those CONS cases that were considered significant pathogens.

3.8.3. Neutropenia, Lymphopenia and Monocytopenia

An absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and absolute monocyte count (AMC) of < 1000 cells/µL was defined as moderate bone marrow suppression, < 500 cells/µL as severe suppression and < 100 cells/µL as profound suppression. Prolonged neutropenia, lymphopenia, and monocytopenia were defined as an ANC, ALC or AMC of < 1000 cells/µL for ≥ 7 days (Freifeld et al., 2011).

3.8.4. Pneumonia

Children who have evidence of crackles in the absence of wheezing, or the presence of any airspace consolidation on radiograph were categorized as having pneumonia.

3.8.5. Treatment Intensity

Treatment intensity 1 (Standard intensity) was protocols used to treat localised solid tumours, treatment intensity 2 (Medium intensity) was for metastatic solid tumours and medium-risk ALL, and treatment intensity 3 (High intensity) was used for AML, high-risk ALL and stem-cell transplantation.

3.9. Investigations on Admission

3.9.1. Nutritional Status at Presentation

3.9.1.1. Anthropometry

Anthropometry was assessed by measuring:

- Height in centimetres
- Weight in kilograms
- Mid-upper arm circumference in millimetres
- Triceps skin fold thickness in millimetres
- WHO-Anthro and WHO-Anthro plus calculators were used to calculate WFA, WFH and HFA scores, BMI and Z-scores.

3.9.1.2. Mid-upper arm circumference

Mid-upper arm circumference was measured to the nearest 1 mm. The mid-point of the dependant right upper arm was determined, between the olecranon process of the ulna and the acromial process of the scapula with the forearm held at a right angle and a mark made at this point. A paper measuring tape was passed around the arm at the mark. The measurement was repeated twice and the mean of the three measurements was used for analysis. The results were compared with age and gender matched norms.

3.9.1.3. TRICEPS SKIN FOLD THICKNESS

A Harpenden calliper (John Bull British Indicators, LTD., made in England) was used to measure the TSFT to the nearest 0.2 mm at the same level of the site used for the MUAC. The skin and fat was lifted away from underlying muscle tissue and the calliper blades were applied to either side of the fold of skin. The measurements were repeated twice within 1mm of the previous measurement and the mean of the three measurements was used for analysis. The results were referenced to age and gender matched norms (Frisancho, 1981).

Mid upper arm circumference and TSFT are surrogate measures of lean muscle and fat. The WFH may be misleading in children with malignancy, especially in children with large STs, and particularly in children with abdominal tumours, which often weigh more than ten percent of the total body weight, and in patients on steroid therapy who may have excessive weight gain. MUAC and TSFT are valuable tools in such patients, they are independent of tumour mass (Sala et al., 2004).We used the TSFT, MUAC and AMA to assess nutritional status in our study.

3.9.2. SCREENING FOR TUBERCULOSIS

3.9.2.1. TUBERCULIN SKIN TESTING

All patients enrolled on the study were screened for MTB infection using the Mantoux method, where 0.1ml of purified protein derivative (23TS), TUBERSOL®, Sanofi Pasteur, Canada) was intradermally injected on the volar aspect of the left forearm. The reactivity to the TST was measured within 48-72 hours. A transverse diameter of $\geq$ 5 millimetres induration was considered to indicate underlying MTB infection, as all the patients were considered immunocompromised.

3.9.2.2. Ellispot Interferon Gamma Release Assay Test

Five millilitres of blood was obtained in a heparinised tube from all children at the time of enrolment for the IGRA test. ELISPOT was performed using a commercial kit (T-SPOT.TB; Oxford Immunotec Ltd, Oxford, UK) (Lalvani et al., 2001) at the Respiratory and Meningeal Pathogens Research Unit, Chris Hani Baragwanath Academic Hospital. The peripheral blood mononuclear cells were separated using Ficoll-Plaque centrifugation. The cells were washed, suspended, and counted. The wells of polyvinylidene fluoride–backed plates (MAIPS4510; Millipore, Billerica, MA, USA) were coated with 15 μg/mL of monoclonal antibody 1-D1K against IFN-γ (Mabtech, Nacka Strand, Sweden). The separated cells (250 000/well) were added to duplicate wells containing antigen (ESAT-6 or CFP-10) or mitogen. The antigen was not added to the background control wells. After an incubation period of 18 hours, the plates were washed, and one μg/mL of biotinylated monoclonal antibody 7-B6-1-biotin against IFN-γ (Mabtech) was added. The plates were incubated for two hours, and then rewashed. Streptavidin-alkaline phosphatase toxoid (Mabtech) was added and incubated for 1.5 hours. The plates were once again rewashed, and 100 μL of chromogenic alkaline phosphatase substrate (Bio-Rad Laboratories, Hercules, CA, USA) was added. After 10–15 min, the plates were once again washed and spots were enumerated with a stereomicroscope. Mean values were determined and both duplicate wells were used in all calculations. The total numbers of spots (background control wells) were subtracted from the number in the test wells, and a response was positive if the number of spots per test well was ≥ 10 and at least twice the value found in the background control wells.

3.9.3. Human Immunodeficiency Virus

The HIV-1 ELISA assays for HIV serology were performed using the HIV Architect (Abbot, Germany, 2006) or HIV Elecys-Cobas (Roche, Sweden, 2008). The HIV Ag/Ab Combo Architect (Abbot, Germany, 2006) used for confirmatory testing of HIV ELISA positivity. Both the assays detect HIV-1 antigen p 24 and total antibodies to HIV-1 and HIV-2. For children younger than eighteen months of age, the HIV-PCR test was done using the HIV PCR/Cobas Ampiclor (Roche, Sweden, 2010).

3.10. INVESTIGATIONS FOR A SEPTIC EPISODE

Patients on treatment for cancer who presented with a pyrexial episode, i.e. a single temperature of ≥ 38.5°C or two temperature recordings of ≥ 38.0 C within a 24-hour period were enrolled into the sepsis component of the study. Each patient had a thorough septic work-up done as per Appendices 3, 6 and 7.

3.11. DESCRIPTION OF TESTS

3.11.1. FULL BLOOD COUNT AND DIFFERENTIAL

The full blood count was run on the Sysmex XE-5000 Analyser (Sysmex, Kobe, Japan, 2009). The Sysmex XE-5000 reports the white blood cell, red blood cell, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, red cell distribution width, platelet, mean platelet volume, and plateletcrit. The five-part differential includes percent and absolute number of neutrophils, lymphoid cells, monocytes, eosinophils, and basophils.

3.11.2. UREA, ELECTROLYTES AND ALBUMIN

The test was performed using the colorimetric method using bromocresol green (BCG), Roche Modular P and Cobas 8000 (Roche Diagnostics GmbH, Mannheim, Germany, 2006).

3.11.3. C-REACTIVE PROTEIN

The test was performed using particle enhanced immune-turbidimetric method using the Roche Modular P and Cobas 8000 (Roche, Germany, 2006).

3.11.4. Procalcitonin

The test was performed using electro-chemiluminescent method using the Roche Modular E and Cobas 8000 (Roche, Germany, 2006).

3.11.5. Blood Culture

Blood cultures were processed using the BacT/ALERT 3D, non-invasive microbial detection system (BioMerieux, France, 2005). The instrument is automated, capable of incubating, agitating, and continuously monitoring aerobic and anaerobic media inoculated with patient specimens suspected of having bacteraemia and fungaemia.

Carbon dioxide determines the positivity of the culture bottle and substrates in the culture medium. As the microorganisms metabolize the substrates in the culture medium, CO- CO_2 is produced which causes the colour of the sensor at the bottom of the bottle, to change from dark to light. A light-emitting diode projects light onto the sensor and a photodetector measures the reflected light. The amount of CO_2 produced determines the positivity of the culture bottle.

3.11.6. Antibiotic Susceptibility testing

Isolates from blood culture, urine and other sterile sites (if applicable) investigated for antibiotic susceptibility to pre-defined antibiotic compounds either manually or using the automated instrument following the Kirby-Bauer method, and interpretation of susceptibility was based on the CLSI standards M100-S19-24 (Microscan, USA, 2006).

3.12. Investigation for TB Disease

3.12.1. Microscopy and Culture

Sputum specimens were collected for microscopy and culture in patients suspected of having TB disease. In those patients who were unable to expectorate a sputum specimen, a gastric lavage specimen was obtained. The gastric lavage was performed by placing an appropriate-sized nasogastric tube trans-oesophageally in the stomach of a fasting patient early in the morning, before arising. The stomach was washed with normal saline, followed by aspiration of the gastric contents. The sample was collected in a sterile bottle and delivered to the laboratory. Samples were collected on three consecutive mornings. Trained nursing personnel collected the sputum. The mouth was first rinsed to avoid contamination with food particles and mouth flora. The patient was instructed to inhale deeply and cough deeply from the chest. We targeted obtaining a minimum of 2 ml of sputum.

3.13. Respiratory virus testing

Nasopharyngeal aspirates (NPAs) were collected on all patients suspected of having a septic episode, except for cases diagnosed from Friday evening to Sunday (due to logistical issues) and in patients with epistaxis. Nasal secretion samples were obtained using a nasogastric tube (FG 8 x 10 cm in length, Given Medical Products, South Africa), attached to a 5 ml syringe containing 3 ml of normal saline. The nasopharynx was injected with saline and one to two millilitres was immediately aspirated. The NPA was placed in a universal transport medium and stored at 4°C. As soon as possible, this was then transported to the Respiratory and Meningeal Pathogens Research Unit, Chris Hani Baragwanath Academic Hospital.

Total nucleic acids were extracted from archived NPA using a Nucli-SENS easy MAG platform (BioMerieux; France, 2009), and eluted in a final volume of 60 ml of elution buffer (Loens et al., 2007). RNA was reverse transcribed with High Capacity cDNA Reverse Transcriptase (Invitrogen, Life Technologies, USA) and primed with oligo-dT primers (Invitrogen, Life Technologies; USA). RT- PCR was done in an ABI 7500 RT-PCR system

(Applied Biosystems, Life Technologies; USA); reactions were performed in 20 ml using TaqMan Universal PCR Master Mix (Applied Biosystems, Life Technologies; USA) and the primers and probes listed in Appendix 7. Five duplex RT-PCR reactions, targeting eight respiratory viruses were developed. Internal controls (the human genes: ribonucleoprotein and glyceraldehyde-3-phosphate dehydrogenase; or the spiked viruses: lambda and Newcastle Disease Virus) were included to check the efficiency of the extraction step and to detect the presence of PCR inhibitors. Positive controls were included in each experiment (Nunes et al., 2014).

4. Bacterial And Fungal Infections in Children Treated for Cancer

4.2. Results

4.2.1. Demographic and Clinical Data of Children with Cancer

We enrolled 169 patients into the study, of whom 82 (42.5%) were diagnosed with a haematological malignancy (HM) and 87 (51.5%) with a solid tumour (ST). The HM cases included 32 (38.6%) acute lymphoblastic leukaemia (ALL), 10 (12%) acute myeloid leukaemia (AML), 23 (27.7%) Non-Hodgkin's lymphoma (NHL) and 18 (21.7%) Hodgkin's lymphoma (HL). Among the 87 patients with ST, 29 (33.7%) had Wilm's tumour (WT), 13 (15.1%) rhabdomyosarcoma (RMS), nine (10.5%) each of retinoblastoma (RBL) and brain tumours (BT), five (5.8%) each of Kaposi's sarcoma (KS) and osteogenic sarcoma (OS), four (4.7%) each of neuroblastoma (NBL) and germ cell tumours (GCT), three (3.5%) of hepatoblastomas (HBL), two (2.3%) of nasopharyngeal carcinomas (NPC) and three (3.5%) children with rare tumours (two adrenocortical carcinomas, one hepatocellular carcinoma).

Overall, 56.8% of the children were males, including being more commonly represented among HM (68.3%) than ST (45.9%) cases;

Table 1 (p=0.006). The median age of presentation was older for children with HM (89 months) than those with ST (48 months); Table 1.

Twenty-five of the patients with ST had Stage 4 disseminated disease (6 RMS, 4 WT, and 1 RBL). Of the 18 patients with HL, ten had extra-nodal disease spread to the bone marrow. Seventeen (73.9%) of the patients with NHL had high-risk disease. One patient with NHL required second-line chemo-therapy after relapse and demised prior to autologous stem cell transplant. One HIV-infected patient was treated with an autologous stem cell transplant post relapse of disease.

Nine (28.1%) of the 32 patients with ALL failed induction therapy, therefore needed a second-line course of induction chemotherapy. Twenty-five (78.1%) of the ALL were stratified as high-risk patients. Ten (6.3%) patients were treated with autologous stem cell transplantation (two each of RMS, HL, NBL, NHL, and one each with WT and OS). Eleven children (6.5%) were not treated (eight abandoned treatment and three were not treated because of advanced disease). These children were included in the analysis until death or abandonment of treatment.

Table 1: Demographic and Clinical Data of Children with Cancer

Characteristic	Overall N=169	Haematological Malignancy N=82	Solid Tumour N=87	p-value*
Total	169	82 (42.5%)	87 (51.5%)	-
Male	96 (56.8%)	56 (68.3%)	40 (45.9%)	**0.006**
Median Age (IQR[1])-months	68.5 (36-121)	89 (51-23)	48 (27.5-100)	**0.001**
HIV-Infected	18 (10.6%)	13 (15.9%)	5 (5.8%)	**0.06**

N= number of patients, IQR[1] = Interquartile range,*p= Haematological Malignancy vs Solid Tumour

4.1.2. Aetiology Of Microbiologically Confirmed Septic Episodes in Children treated for cancer

One hundred and thirty-one (77.5%) of the 169 patients enrolled in the study experienced at least one microbiological confirmed sepsis episode (MCSE) during treatment for cancer, which was more common among children with HM (87.8%) compared to those with ST (67.8%; p=0.003); Table 2. The mean number of suspected sepsis episodes (SSE) was 3.1 per patient, which also was more common among those with HM (4.0) than in children with ST (2.3; p<0.001); Table 2.

The person-time of follow-up was 234 and 279 child years among the HM and ST cases, respectively; Table 2. There was a higher incidence (per 100 child years) of SSE among children with HM (140) than in those with ST (71; $p<0.001$); Table 3. Overall, the incidence (per 100 child years) of MCSE was 69.4, which too, was higher among children with HM (96.7) compared to those with ST (46.5; $p<0.001$); Table 2. Overall there were 3.1 septic episodes per patient, 4 in those with HM and 2.3 ($p<0.001$) in the cohort with ST. Similarly, the incidence of Gram-positive bacteraemia (77.0 vs 43.7; $p<0.001$) and Gram-negative bacteraemia (74.5 vs 25.8; $p<0.001$) were greater in children with HM than ST; Table 3. In addition, the incidence of concurrent Gram-positive and Gram-negative bacteraemia (27.8 vs 11.1; $p<0.001$) were higher in children with HM than ST.

Table 2: Aetiology of Microbiologically Confirmed Septic Episodes in Children Treated for Cancer

Characteristic	Overall N[1] = 169	Haematological Malignancy N = 82	Solid Tumour N = 87	P-value*
Person time observation (years)	513	234	279	NA
Number of children with at least one SSE[3] (%)	131 (77.5)	72 (87.8)	59 (67.8)	**0.003**
Total number of SSE	528	330	198	NA
Mean number of SSE per patient (SD)[4]	3.1 (3.1)	4.0 (3.44)	2.3 (2.4)	**<0.001**
SSE episodes per 100 child years (95% CI [5])	102.9 (94.1-111.7); n[2] =528	141.2 (126-156.5); n=330	70.9 (61-80.7); n=198	**<0.001**
At least one positive blood culture/patient (%)	120 (71.0)	69 (84.2)	51 (58.6)	**<0.001**
At least one episode of GPB[6] (%)	109 (64.5)	63 (76.8)	46 (52.9)	**0.002**
At least one episode of GNB[7] (%)	86 (50.9)	53 (64.6)	33 (37.9)	**0.001**
At least one episode of Fungaemia (%)	36 (21.3)	20 (24.4)	16 (18.4)	0.445
Total number of GPB Septic Episodes	302	180	122	NA[10]
Total number of GNB Septic Episodes	246	174	72	NA
Total number of Fungal Septic Episodes	47	24	23	NA
Incidence of MCSE[7] per 100 child year (95% CI)	69.4 (62.2-76.6); n=356	96.7 (84.1-109.3); n=226	46.5 (38.5-54.5); n=130	**<0.001**
Incidence of Gram-positive bacteraemia per 100 child years (95% CI)	58.9 (52.2-65.5); n=302	77 (65.8-88.3); n=180	43.7 (35.9-51.4); n=122	**<0.001**

Incidence of Gram-negative bacteraemia per 100 child years (95% CI)	47.9 (42-53.9); n=246	74.5 (63.4-85.5); n=174	25.8 (19.8-31.7); n=72	**<0.001**
Incidence of Fungaemia per 100 child years (95% CI)	9.2 (6.5-11.8); n=47	10.3 (6.2-14.4); n=24	8.2 (4.9-11.6); n=23	0.473
Incidence[9] of Gram-positive and Gram-negative bacteraemia per 100 child years (95% CI)	18.7 (15-22.5); n=96	27.8 (21.1-34.6); n=65	11.1 (7.2-15); n=31	**<0.001**
Incidence of Gram-positive bacteraemia + Fungaemia per 100 child years (95% CI)	4.5 (2.7-6.3); n=23	3.9 (1.3-6.4); n=9	5 (2.4-7.6); n=14	0.541
Incidence of Gram-negative bacteraemia +Fungaemia per 100 child years (95% CI)	9 (6.4-11.6); n=46	9.8 (5.8-13.9); n=23	8.2 (4.9-11.6); n=23	4

N[1]=number of patients, n[2]=number of events, SSE[3] = Suspected Septic Episode, SD[4] = Standard deviation, CI [5] = Confidence interval, GPB[6] = Gram-positive Bacteria, GNB[7] = Gram-negative Bacteria, MCSE[8] =Microbiologically-Confirmed Septic Episodes, Incidence[9] included in the incidence of Gram-positive and negative bacteraemia and fungaemia, *p= Haematological Malignancy vs Solid Tumour, NA[10 =] not applicable

4.2.2. Clinical and Laboratory Parameters of Suspected and Microbiologically Confirmed Sepsis in Children Treated for Cancer

The mean CRP (p=0.011) and PCT (p=0.001) were higher in children with HM among those with MCSE compared to those with culture-negative sepsis episodes (CNSE); Table 3. The maximum recorded temperature, duration of fever, mean white cell count, neutrophil count, lymphocyte count, and monocyte count showed no significant difference between MCSE compared to CNSE; Table 3. Using MUAC, 76.9% of the children were severely malnourished, whereas by AMA 56.8% were categorized as being severely malnourished. There was, however, no significant relationship between nutritional status on admission for those with HM and those with ST (data not shown).

Table 3: Clinical and Laboratory Parameters of Suspected and Microbiologically Confirmed Sepsis in Children Treated for Cancer

Parameter	Overall N = 169			Haematological Malignancy N = 82			Solid Tumour N = 87		
	CNSE[1]	MCSE[2]	*p-value	CNSE	MCSE	**p-value	CNSE	MCSE	***p-value
Mean maximum temperature °C (SD[3])	38.9 (0.5)	39.1 (0.6)	0.24	38.9 (0.5)	39.1 (0.6)	0.13	38.9 (0.5)	38.9 (0.5)	0.97
Median duration of fever (days) (IQR[4])	3.0 (2.0-5.0)	3.0 (2.0-5.0)	0.12	3.0 (2.0-5.0)	4.0 (3.0-6.0)	0.20	3.0 (2.0-4.0)	3.0 (2.0-4.0)	0.47
Median CRP[5] mg/L (IQR)	99.0 (34.0-193)	112 (47.5-216)	0.18	117 (61.3-225)	129 (72.8-230)	**0.011**	40.0 (20.5-124)	79.5 (35.3-134)	0.08
Median PCT[6] µ/L (IQR)	1.4 (0.5- 6.8)	2.2 (0.7-11.6)	**0.06**	1.8 (0.5- 7.0)	2.7 (0.7-12.3)	**<0.001**	1.2 (0.5-2.9)	2.0 (0.6-8.9)	0.27
Median WCC[7] x 10^9/L (IQR)	1.4 (0.5-4.6)	1.3 (0.4-5.8)	0.43	0.8 (0.2-1.9)	0.6 (0.3-2.4)	0.38	4.7 (2.2-8.5)	4.5 (1.1-9.0)	0.95
Median neutrophil count(cells/µL) (IQR)	155 (70.0-1898)	220 (70.0-2345)	0.29	90.0 (60.0-378)	90.0 (60.0-878)	0.13	2158 (421-4815)	1410 (120.0-4800)	0.82
Median lymphocyte count(cells/µL) (IQR)	330 (90.0-1380)	295 (80.0-1170)	0.77	110 (72.5-552)	90.0 (70.0-603)	0.77	1500 (7195-2520)	960 (160-1780)	0.36
Median monocyte count(cells/µL) (IQR)	80.0 (30.0-410)	80.0 (30.0-490)	0.70	55.0 (27.5-151)	50.0 (20.0-140)	0.98	410 (75.0-1060)	291 (40.0- 805)	0.42

N = number of patients, CNSE[1] = Culture-negative septic episode, MSCE[2] = Microbiologically-confirmed Septic Episode, SD[3] = Standard deviation, IQR[4] = interquartile ratio, CRP[5] = C-reactive protein, PCT[6] = Procalcitonin, WCC[7] = White cell count, *p = CNSE vs MCSE in the overall group, **p = CNSE vs MCSE for HM, ***p = CNSE vs MCSE for ST

4.2.3. Admission Parameters Associated with Microbiologically Confirmed Sepsis

Children with HM had a 2.03 ($p<0.001$) fold greater incidence (80 per 100 child years) of micro-biologically confirmed sepsis (MSCE) compared to those with ST (40); Table 4. The incidence of MCSE in children categorised as having high-risk HM ((109) was 4.01 fold greater compared to those with medium-risk HM (27; $p<0.001$); Table 4. Similarly, treatment intensity categories 2 and 3 were associated with 2.03 and 8-fold greater incidence of MSCE compared to children with treatment intensity 1 ($p<0.001$ for both; Table 4). In children with ST, those with metastatic disease had a higher incidence (71) of MSCE than those with only localized ST (29; aOR: 2.49; $p<0.001$); Table 4. There was no association observed for age group, gender, nutritional status or HIV-infection status and incidence of MCSE; Table 4.

4.2.4. Factors Associations with Microbiologically Confirmed Sepsis in Children with Cancer

The presence of an indwelling catheter was 2.98 fold ($p<0.0001$) more likely to be associated with MCSE in children with SSE compared to those without an indwelling catheter; Table 5. No such associations of a greater proportion of SSE cases having MCSE were evident in the presence of mucositis, herpes stomatitis, use of high-dose corticosteroids and prolonged or profound bone marrow suppression; Table 5.

Table 4: Admission Parameters Associated with Microbiologically Confirmed Sepsis

Parameter	Incidence Rate (95% CI)	Adjusted Incidence Rate Ratio (95% CI; p-value)
Age (years)		
1-5	61.5 (52.2-70.8)	1.14 (0.88-1.48); p= 0.31
>5-10	51.1 (40.8-61.4)	0.83 (0.62-1.11); p= 0.20
>10	61.6 (48.9-74.4)	referent group
Female	57.6 (48.7-66.6)	referent group
Male	59.2 (50.9-67.4)	**0.8 (0.64-1); p= 0.048**
MUAC[1]>5th percentile	56.6 (44-69.3)	referent group
MUAC<5th percentile	59.4 (52.4-66.3)	1.07 (0.83-1.38); p= 0.59
AMA[2]>5th percentile	55.5 (46.5-64.4)	referent group
AMA<5th percentile	61.3 (53-69.6)	1.03 (0.83-1.27); p= 0.79
TSFT[3]>5th percentile	61.8 (53.9-69.7)	referent group
TSFT<5th percentile	53.6 (44-63.2)	0.9 (0.73-1.13); p= 0.37
HIV-uninfected	59.2 (52.8-65.7)	referent group
HIV-infected	50.9 (32.4-69.4)	0.73 (0.5-1.07); p= 0.10
Solid Tumour	39.6 (32.8-46.4)	referent group
Solid Tumour (localized)	28.7 (22-35.4)	referent group
Solid Tumour (metastatic)	71.4 (53.4-89.5)	**2.49 (1.76-3.51); p<0.001**
Haematological malignancy	80.4 (69.9-90.9)	**2.03 (1.64-2.52); p<0.001**
Medium risk	27.3 (17-37.6)	referent group
High risk	109.3 (94.1-124.5)	**4.01 (2.68-5.99); p<0.001**
Treatment intensity[4] 1	26.1 (20-32.2)	referent group
Treatment intensity 2	47.5 (37.4-57.6)	**2.03 (1.47-2.79); p<0.001**
Treatment intensity 3	124.1 (106.9-141.2)	**8 (5.24-12.2); p<0.001**

MUAC [1] = Mid-upper arm circumference, AMA [2] = Arm muscle area, TSFT [3] = Triceps skin-fold thickness, Treatment Intensity [4] = Treatment Intensity 1 = standard intensity, 2 = medium intensity, 3= high intensity, CI [5] = Confidence interval

Table 5: Association between Suspected and Microbiologically Confirmed Sepsis in Children Treated for Cancer

Parameter	Proportion with MCSE[4] (95% CI[5])	Adjusted Odds Ratio (95% CI; p-value)
No Indwelling Catheters	46.7 (36.4-57.4)	referent group
Indwelling Catheters	71.7 (67.2-75.9)	**2.9 (1.9-4.8); p<0.001**
No high-dose Corticosteroids	64.4 (58.8-69.7)	referent group
High-dose Corticosteroids	71.7 (65.0-77.6)	1.5 (0.9-2.4); p= 0.13
No Mucositis	67.1 (62.2-71.6)	referent group
Mucositis	68.5 (59.6-76.3)	1.03 (0.7-1.6); p= 0.89
No Herpes stomatitis	66.6 (61.8-71.1)	referent group
Herpes stomatitis	70.3 (61.1-78.2)	1.2 (0.8-1.9); p= 0.44
No Profound neutropenia at onset of SSE[1]	67.1 (61.4-72.4)	referent group
Profound [2] Neutropenia at onset of SSE	67.8 (61.4-73.6)	0.9 (0.6-1.5); p= 0.86
No Prolonged Neutropenia during SSE	68 (62.1-73.4)	referent group
Prolonged [3] Neutropenia during SSE	66.8 60.6-72.5)	0.9 (0.6-1.3); p= 0.60
No Profound Lymphopenia at onset of SSE	66.8 (61.3-71.8)	referent group
Profound Lymphopenia at onset of SSE	68.8 (61.9-74.9)	1.04 (0.7-1.6); p= 0.84
No Prolonged Lymphopenia during SSE	66.3 (59.9-72.1)	referent group
Prolonged Lymphopenia during SSE	68.4 (62.6-73.7)	1.05 (0.7-1.6); p= 0.79
No Profound Monocytopenia at onset of SSE	67.1 (60.4-73.2)	referent group
Profound Monocytopenia at onset of SSE	67.9 (62.3-72.9)	0.9 (0.6-1.5); p= 0.86
No Prolonged Monocytopenia during SSE	65.6 (58.1-72.4)	referent group
Prolonged Monocytopenia during SSE	68.6 (63.4-73.4)	1.1 (0.7-1.7); p= 0.8

SSE[1] = Suspected Septic Episode, Profound[2] = Absolute cell count < 100 cells/µL, Prolonged[3] = Absolute cell count <1000 cells/µL for > 7 days, MCSE[4] = microbiologically confirmed septic episode, CI[5] = Confidence interval

4.2.5. Gram-Positive Bacterial Pathogens Causing Sepsis in Children with Cancer

Overall, there were 21.2 Gram-positive MCSE per 100 child years. The most frequently identified Gram-positive bacterial isolates were CONS (n=176; 59.1%), *Streptococcus viridans* (n=36; 12.1%), and *Enterococcus faecium* (n=27, 9.1%). The overall incidence (per 100 child years) of CONS was 34.3, which was higher among children with HM (44.5) than with ST (25.8; p=0.002). Similarly, there was a higher incidence of *Staphylococcus aureus* (3.9 vs 1.8; p=0.026), *Streptococcus pneumoniae* (3.9 vs 0; p=0.002), and *Micrococcus* (3.9 vs 1.1; p=0.044) sepsis episodes among children with HM than with ST; Table 6.

4.2.6. Gram-Negative Bacterial Pathogens Causing Sepsis in Children treated for Cancer

Overall, there were 16.8 Gram-negative MCSE per hundred child years. The most frequently identified Gram-negative bacterial isolates were *Escherichia* species (n=64; 26.9%) and *Acinetobacter* species (38; 16%), and *Klebsiella* species (28; 11.8%). The overall incidence (per 100 child years) of *Escherichia* species was 12.5, which was higher among children with HM (17.5) than ST was (8.2; p=0.005). Similarly, there was a higher incidence of *Acinetobacter* species (12.8 vs. 2.9; p<0.001), *Klebsiella* species (8.1 vs. 3.2; p=0.022), *Pseudomonas* species (8.1 vs. 2.9; p=0.012), and *Bacillus* species (6.4 vs 1.1; p=0.002), and *Stenotrophomonas maltophilia* (4.3 vs 0.7; p=0.01) sepsis episodes among children with HM than ST; Table 7.

4.2.7. Fungal Pathogens Causing Sepsis in Children with Cancer

Overall, there were 16.8 fungal MCSE per hundred child years. The most frequently identified fungal isolates were *Candida albicans* (n= 28; 62.2%) and *Candida parapsilosis* (n=12; 26.7%). The overall incidence (per 100 child years) of *Candida albicans* was six and three for *Candida parapsilosis,* which did not differ between cases with HM and ST; Table 8.

Table 6: Incidence (per 100 child years) of Gram-Positive Bacterial Pathogens Causing Sepsis in Children with Cancer

Incidence	Overall N=514	Haematological Malignancy N=234	Solid Tumour N=280	p-value*
Total number of Septic episodes with Gram-positive bacteraemia	302	180	122	NA
Incidence** of at least one Gram-positive bacterium (95% CI)	21.2 (17.3-25.2); n=109	27 (20.3-33.6); n=63	16.5 (11.7-21.2); n=46	**0.021**
Incidence of CONS[1] (95% CI)	34.3 (29.2-39.4); n=176	44.5 (36-53.1); n=104	25.8 (19.8-31.7); n=72	**0.002**
Incidence of *S. aureus*[2] (95% CI)	3.5 (1.9-5.1); n=18	5.6 (2.5-8.6); n=13	1.8 (0.2-3.4); n=5	**0.026**
Incidence of *S. pneumoniae* (95% CI)	1.8 (0.6-2.9); n=9	3.9 (1.3-6.4); n=9	0 (0-0); n=0	**0.002**
Incidence of *Streptococcus* species[3] (95% CI)	1.9 (0.7-3.2); n=10	3 (0.8-5.2); n=7	1.1 (0-2.3); n=3	0.13
Incidence of *Streptococcus viridans* (95% CI)	7 (4.7-9.3); n=36	7.3 (3.8-10.7); n=17	6.8 (3.7-9.9); n=19	0.85
Incidence of *Enterococcus faecium*[7] (95% CI)	5.3 (3.3-7.2); n=27	5.6 (2.5-8.6); n=13	5 (2.4-7.6); n=14	0.79
Incidence of *Enterococcus faecalis* (95% CI)	1.9 (0.7-3.2); n=10	2.6 (0.5-4.6); n=6	1.4 (0-2.8); n=4	0.37
Incidence of *Micrococcus* (95% CI)	2.3 (1-3.7); n=12	3.9 (1.3-6.4); n=9	1.1 (0-2.3); n=3	**0.044**

CI[1] = Confidence interval, CONS[2] includes Coagulase-negative *Staphylococcus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus hominis*, *S aureus* [3] includes Methicillin-resistant *Staphylococcus aureus*, *Streptococcus* species[4] includes *Streptococcus milleri*, *Streptococcus mitis*, and *Streptococcus salivarius*, *Enterococcus faecium*[5] includes Vancomycin-resistant *Enterococci*, N[6] =Total person years, n[7] = Number of events, NA[8]=Not Applicable, *p value = Haematological Malignancy vs Solid Tumour, Incidence** per 100 child years

Table 7: Incidence (per 100 child years) of Gram-Negative Bacterial Pathogens Causing Sepsis in Children with Cancer

Incidence	Overall N=514	Haematological Malignancy N=234	Solid Tumour N=280	p-value*
Total number of SE with Gram-negative bacteraemia	246	174	72	NA
Incidence** of at least one GNB (95% CI)	16.8 (13.2-20.3); n=86	22.7 (16.6-28.8); n=53	11.8 (7.8-15.8); n=33	**0.006**
Incidence of *Escherichia coli*[1] (95% CI)	12.5 (9.4-15.5); n=64	17.5 (12.2-22.9); n=41	8.2 (4.9-11.6); n=23	**0.005**
Incidence of *Acinetobacter baumanni*[2] (95% CI)	7.4 (51-9.8); n=38	12.8 (8.2-17.4); n=30	2.9 (0.9-4.8); n=8	**<0.001**
Incidence of *Klebsiella* species[4] (95% CI)	5.5 (3.4-7.5); n=28	8.1 (4.5-11.8); n=19	3.2 (1.1-5.3); n=9	**0.022**
Incidence of *Pseudomonas* species[3] (95% CI)	5.3 (3.3-7.2); n=27	8.1 (4.5-11.8); n=19	2.9 (0.9-4.8); n=8	**0.012**
Incidence of *Bacillus* species (95% CI)	3.5 (1.9-5.1); n=18	6.4 (3.2-9.7); n=15	1.1 (0-2.3); n=3	**0.002**
Incidence of *Enterobacter* species[5] (95% CI)	2.7 (1.3-4.2); n=14	3.9 (1.3-6.4); n=9	1.8 (0.2-3.4); n=5	0.17
Incidence of *Corynebacterium* species (95% CI)	2.5 (1.2-3.9); n=13	3.9 (1.3-6.4); n=9	1.4 (0-2.8); n=4	0.09
Incidence of *Stenotrophomonas maltophilia* (95% CI)	2.3 (1-3.7); n=12	4.3 (1.6-6.9); n=10	0.7 (0-1.7); n=2	**0.01**
Incidence of *Neisseria* species (95% CI)	0.8 (0-1.5); n=4	0.9 (0-2); n=2	0.7 (0-1.7); n=2	0.86
Incidence of *Aeromonas caviae* (95% CI)	0.6 (0-1.2); n=3	0.9 (0-2); n=2	0.4 (0-1.1); n=1	0.47
Incidence of *Haemophilus influenza* (95% CI)	0.4 (0-0.9); n=2	0.9 (0-2); n=2	0 (0-0); n=0	0.19
Incidence of *Brevudimonas* species (95% CI)	0.4 (0-0.9); n=2	0.9 (0-2); n=2	0 (0-0); n=0	0.19
Incidence of *Proteus mirabilis* (95% CI)	0.6 (0-1.2); n=3	0.9 (0-2); n=2	0.4 (0-1.1); n=1	0.47

Incidence of *Salmonella* species (95% CI)	0.4 (0-0.9); n=2	0 (0-0); n=0	0.7 (0-1.7); n=2	0.32
Incidence of *Empedobacter* species (95% CI)	0.2 (0-0.6); n=1	0.4 (0-1.3); n=1	0 (0-0); n=0	0.41
Incidence of *Moraxella catarrhalis* (95% CI)	0.2 (0-0.6); n=1	0 (0-0); n=0	0.4 (0-1.1); n=1	0.56
Incidence of *Orchobacter anthropi* (95% CI)	0.2 (0-0.6); n=1	0.4 (0-1.3); n=1	0 (0-0); n=0	0.41
Incidence of *Pantoea* species (95% CI)	0.2 (0-0.6); n=1	0.4 (0-1.3); n=1	0 (0-0); n=0	0.41
Incidence of *Serratia odifera* (95% CI)	0.2 (0-0.6); n=1	0.4 (0-1.3); n=1	0 (0-0); n=0	0.41
Incidence of *Raltsonia picketii* (95% CI)	0.4 (0-0.9); n=2	0.9 (0-2); n=2	0 (0-0); n=0	0.19
Incidence of *Morganella morganella* (95% CI)	0.2 (0-0.6); n=1	0 (0-0); n=0	0.4 (0-1.1); n=1	0.56

CI [1] = Confidence interval, *Escherischia coli* [2] includes ESBL *Escherischia coli, Acinetobacter* species [3] includes *baumanni, lwoffii* and pan-resistant species, *Klebsiella* species [4] includes *baumanni, oxytoca* and ESBL *Klebsiella*, *Pseudomonas* species[5] includes *aeruginosa, alcaligenes, flourescens* and ESBL *Pseudomonas, Enterobacter* species[6] includes *cloacae, agglomerans* and ESBL *Enterobacter,* N [7]= Total person-years, n [8]= number of events, *p= Haematological malignancy vs Solid Tumour. Incidence** per 100 child years

Table 8: Incidence (per 100 child years) of Fungaemia in Children with Haematological and Solid Tumour Malignancies

Parameter	Overall N[2]=514	Haematological Malignancy N=234	Solid Tumour N=280	p-value*
Total number of fungaemia episodes	246	174	72	NA [4]
Incidence** of at least one fungus (95% CI [1])	16.8 (13.2-20.3); n [3]=86	22.7 (16.6-28.8); n=53	11.8 (7.8-15.8); n=33	**0.006**
Incidence of *C. albicans* fungaemia (95% CI)	5.5 (3.4-7.5); n=28	6 (2.9-9.1); n=14	5 (2.4-7.6); n=14	0.65
Incidence of *C. parapsilosis* fungaemia (95% CI)	2.3 (1-3.7); n=12	3 (0.8-5.2); n=7	1.8 (0.2-3.4); n=5	0.38
Incidence of *C. tropicalis* fungaemia (95% CI)	0 (0-0); n=0	0 (0-0); n=0	0 (0-0); n=0	0.93
Incidence of *Prototheca* fungaemia (95% CI)	0.4 (0-0.9); n=2	0.4 (0-1.3); n=1	0.4 (0-1.1); n=1	0.90
Incidence of Fusarium fungaemia (95% CI)	0.2 (0-0.6); n=1	0.4 (0-1.3); n=1	0 (0-0); n=0	0.41
Incidence of *Aspergillus niger* fungaemia (95% CI)	0.2 (0-0.6); n=1	0.4 (0-1.3); n=1	0 (0-0); n=0	0.41
Incidence of *Aspergillus flavus* fungaemia (95% CI)	0.2 (0-0.6); n=1	0 (0-0); n=0	0.4 (0-1.1); n=1	0.56

CI [1]= Confidence interval, N [2]= total person-years, n [3]= number of events, NA 4=Not applicable, p*= Haematological malignancy vs Solid tumour, Incidence** per 100 chi

4.2.8. ANTIMICROBIAL SUSCEPTIBILITY OF GRAM-POSITIVE BACTERIA ISOLATED DURING SEPSIS IN CHILDREN TREATED FOR CANCER

The *Enterococcus* isolates were generally fully sensitive to Linezolid, Teicoplanin, and Quinopristin-dalfopristin; Figure 1. However, 65% of *Enterococcu*s *faecium* species were resistant to Vancomycin. Resistance of *Enterococcus* isolates was > 85% to penicillin and 62% of *Enterococcus faecium* strains were resistant to Ampicillin. The prevalence of resistance of *Staphylococcus aureus* strains was 100% for penicillin-ampicillin, whilst 25% were methicillin resistant. *Staphylococcus aureus* was 100% sensitive to Amikacin, 75% to Clindamycin, 76 % to Trimethoprim-Sulfamethoxazole, 65% to Rifampicin and 68% to Ciprofloxacillin. Not all the GPB were tested against all the antibiotics. *Streptococcus pneumoniae* was fully sensitive to Cefotaxime.

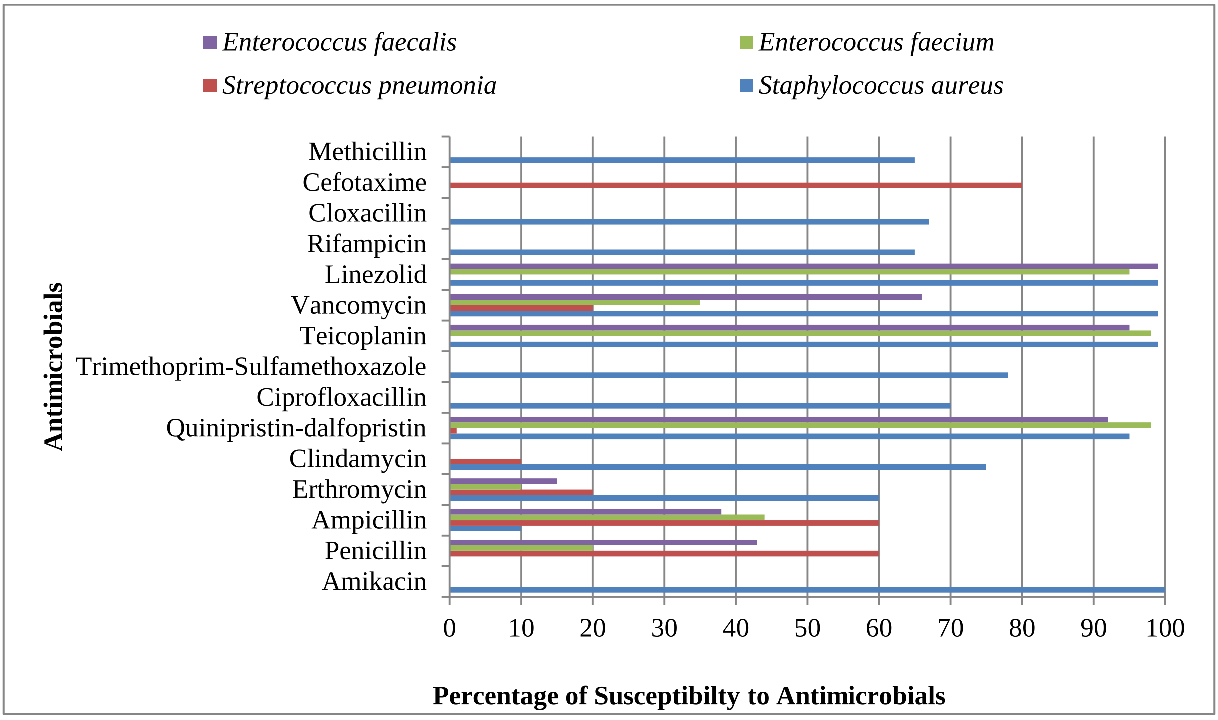

Figure 1: Antimicrobial Susceptibility of Gram-Positive Bacterial Pathogens Causing Sepsis in Children with Cancer

4.2.9. ANTIMICROBIAL SUSCEPTIBILITY OF GRAM-NEGATIVE BACTERIAL PATHOGENS CAUSING SEPSIS IN CHILDREN TREATED FOR CANCER

The antibiotic susceptibility profile of the five most common GNB causing sepsis is shown in Figure 2. The majority of Gram-negative pathogens were susceptible to the Carbapenems except for *Acinetobacter baumanni* (25%-30% sensitivity) and *Pseudomonas aeruginosa* (72% sensitivity). *Escherichia coli* were resistant to 49% - 64% of third generation Cephalosporins. *Klebsiella pneumoniae* was resistant to 65%-78% third generation Cephalosporins. *Pseudomonas aeruginosa* isolates showed less resistance to the antimicrobials tested than most of the other Gram-negative pathogens, 65% of the isolates were sensitive to Piperacillin-Tazobactam, 76% to Ceftazidime, 75% to Amikacin, and 72% and 71% sensitive to Meropenem and Imipenem, respectively. A*cinetobacter baumanni* was resistant to most antimicrobials tested, 27% of the isolates were sensitive to Piperacillin-Tazobactam, 26% to Ceftazidime, 50% to Amikacin, and 26% and 30% sensitive to Meropenem and Imipenem, respectively. *Acinetobacter baumanni* and *Pseudomonas aeruginosa* were not tested against Ertapenem.

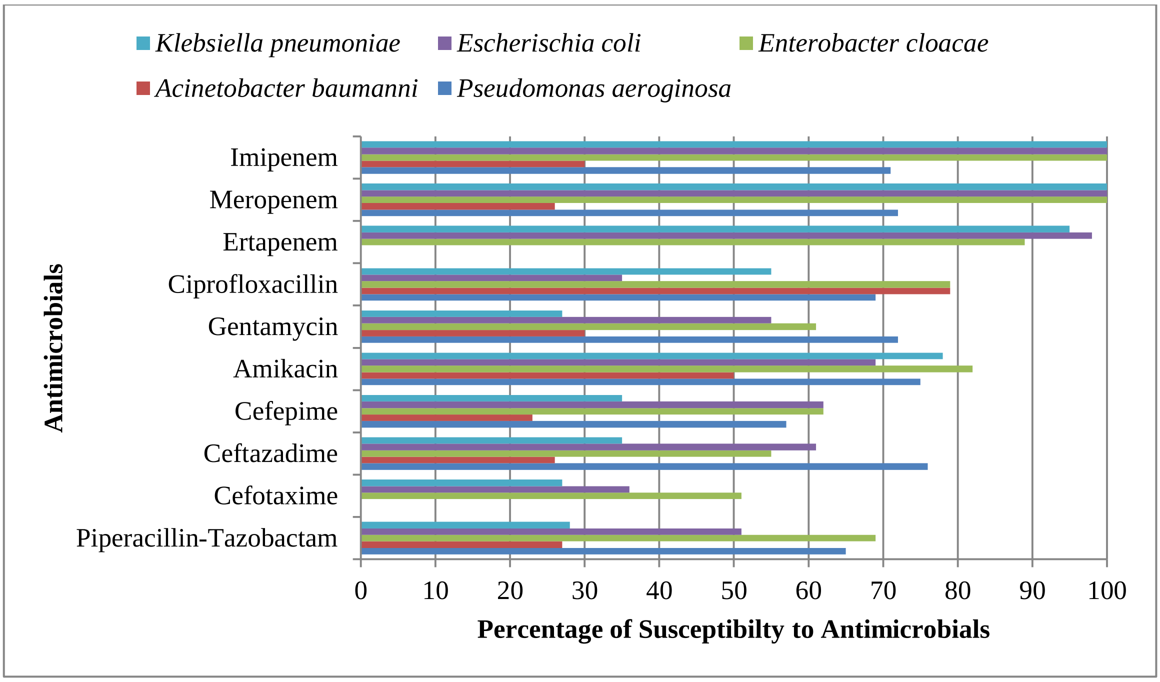

Figure 2: Antimicrobial Susceptibility of Gram-Negative Bacterial Pathogens Causing Sepsis in Children Treated for Cancer

4.2.10. OUTCOME

Of the 169 children enrolled in the study, 53 (31.4%) died. Of these, 13 (7.6%) of the deaths were due to sepsis. Of the patients who died due to sepsis, eleven (84.6%) had pancytopenia during the septic episode. All of the sepsis-associated deaths occurred among children with HM (seven ALL, four NHL, one each of AML and HL). In the cohort with HM, 15.9% of deaths were due to sepsis. Of the thirteen children who died from sepsis, six children with ALL were stratified as high-risk cases and two had failed front-line induction therapy. Of the children with ALL, three deaths were during the induction phase, two after high-risk blocks,one during consolidation, and one during re-induction of the ALL-BFM 1995 protocol.

All four (100%) of the patients with NHL who died of sepsis, were stratified as high-risk, and were HIV-infected. Eight (15.1%) of the 53 who died were HIV-infected, four (30.8%) of the HIV-infected children in our cohort died because of sepsis. *Aspergillus niger* was isolated from blood culture, and an ESBL *Klebsiella pneumoniae* from the urine, in the patient with HL who died post-autologous bone marrow transplant.

Among the sepsis related deaths, 10 (76.9%) were associated with pneumonia, including four (30.8%) children with concurrent microbiologically confirmed TB. Four (30.8%) deaths had a fungal infection, including one each with confirmed *Candida albicans* endocarditis and *Aspergillus* pneumonia. Five (38.5%) of the children who died also had a urinary tract infection in which bacteria were identified. Eight (61.5%) patients had septic shock at the time of death and were not accepted to the Intensive Care Unit for mechanical ventilation; Table 9.

Table 9: Summary of Deaths in Children with Cancer due to Sepsis

Pt	Sex	Age	Disease	HIV	Blood culture	Urine culture	Other site	Clinical Syndrome
1	M	158	NHL[1]	Pos	MRSA[5] CONS[6]	Negative	CSF[9]: MRSA Skin: MRSA	Mucositis
2	M	139	HL[2]	Neg	*Bacillus*, *Enterococcus faecalis*,VRE[7]*Aspergillus niger*	*Ent faecalis*, ESBL *E coli*	Nil	Pneumonia, Gastroenteritis Herpes stomatitis, Mucositis
3	M	137	ALL[3]	Neg	CONS	ESBL *E coli* *C albicans*	Nil	Urinary tract infection
4	M	125	ALL	Neg	*Bacillus*, *Klebsiella Pneumonia*	No growth	Sputum: MTB[10]	Pneumonia, Herpes stomatitis, Proven TB[11]
5	F	112	ALL	Neg	*Acineto baumanni*, *Emped brevis*	ESBL *Pseudo aeruginosa*	Nil	Pneumonia, Gastroenteritis, Urinary tract infection
6	F	100	NHL	Pos	Negative	Negative	Sputum: MTB	Pneumonia, Herpes stomatitis, Mucositis, Proven TB
7	F	99	ALL	Neg	*Candida Albicans*	No growth	Nil	Mucositis

8	F	98	ALL	Neg	ESBL[8] *Klebsiella pneumonia*	Negative	Sputum: MTB	Pneumonia, Gastroenteritis, Proven TB
9	M	50	NHL	Pos	CONS	*E coli*	Nil	Pneumonia, Gastroenteritis, Herpes stomatitis, Mucositis, Urinary tract infection, Probable TB
10	F	44	ALL	Neg	Negative	ESBL *Klebs pneumonia*	*Candida albicans*	Pneumonia, Herpes stomatitis, Fungal urinary tract infection
11	M	23	NHL	Pos	ESBL *E coli*	Nil	Sputum : MTB	Herpes stomatitis, Mucositis
12	M	21	AML[4]	Neg	*Ent faecalis*	*Ent faecalis*	Nil	Pneumonia, Gastroenteritis, Herpes stomatitis, Mucositis, Probable TB

NHL[1] = Non-Hodgkin's lymphoma, HL[2]= Hodgkin's lymphoma, ALL[3] = Acute lymphoblastic leukaemia, AML[4] = Acute myeloid leukaemia, MRSA[5] = Methicillin-resistant *Staphylococci*, CONS[6] = Coagulase-negative *Staphylococci*, VRE[7] = Vancomycin-resistant *Enterococci*, ESBL[8] = Extended-spectrum β lactamase producer, CSF[9] = Cerebrospinal fluid, MTB[10] = Mycobacterium tuberculosis, TB[11] = Tuberculosis

4.2.11. Baseline Parameters Associated with All-cause Mortality

Compared to the mortality rate in children >10 years of age (55.6%), those aged <5 years (28.8%; p=0.012) or 5-10 years (34.0%, p=0.039) had lower mortality rates; Table 10. Overall, children with a HM had a higher case fatality ratio (CFR; 47.6%) compared to those with ST (27.6%; aOR 2.3; p=0.015). Furthermore, the CFR among HM cases with high-risk disease (59.7%) was 11.9 fold greater compared to those with medium risk disease (10%; p=0.02). There was no association between presence of malnutrition or HIV positivity and death in our cohort; Table 10.

4.2.12. Factors Associated with All-Cause Mortality in Children treated for Cancer

The CFR among children who received at least one course of high-dose corticosteroids (58.6%) was 4.9 fold greater compared to those who did not receive high-dose corticosteroids (21.4%; p=0.007). In addition, CFR was 7.8 fold higher among children treated with high-intensity treatment (63.8%) compared to those treated with standard and medium intensity treatment (p=0.004); Table 11. Additional factors independently associated with a higher CFR included developing pneumonia (51.5% vs 27.7%; aOR 2.4; p=0.021) and being diagnosed with tuberculosis (66.7% vs 33.6%; aOR 3.7; p=0.009);

Table 11.

Neither the diagnosis of at least one SSE or MCSE were independently associated with mortality among our study cohort; Table 11. However, the odds of dying in those who had profound neutropenia at the onset of MCSE was 6.7 fold greater (p<0.001), and those with prolonged neutropenia 5.5 fold greater (p=0.003) compared to those sepsis episodes in which this was not present. Likewise, the odds of dying in those who had profound lymphopenia at the onset of MCSE was 5.4 (p=0.001) greater, and those with prolonged lymphopenia were 2.8 fold more likely to die (p=0.048) compared to MCSE cases in which this was not evident.

Table 10: Baseline Parameters Associated with All-cause Mortality

Parameter	Percentage of death (95% CI)	Adjusted Odds Ratio (95% CI; p-value)
Age (years)		
<5	28.8 (19.1-40.7)	**0.4 (0.2-0.8); p= 0.012**
>5-10	34 (21.6-48.9)	**0.4 (0.2-0.9); p= 0.039**
>10	55.6 (40.1-70.1)	referent group
Female	31.5 (21.4-43.6)	referent group
Male	41.7 (31.8-52.2)	1.3 (0.7-2.6); p= 0.41
MUAC[1]>5th percentile	36.1 (21.3-53.8)	referent group
MUAC<5th percentile	36.9 (28.8-45.9)	1.1 (0.5-2.3); p= 0.9
AMA[2]>5th percentile	31.4 (21.2-43.8)	referent group
AMA<5th percentile	40.6 (30.9-51.2)	1.4 (0.7-2.7); p= 0.34
TSFT[3]>5th percentile	33.7 (24.8-43.8)	referent group
TSFT<5th	41.5 (29.7-54.4)	1.5 (0.8-2.9); p= 0.25
HIV-uninfected	36.4 (28.9-44.7)	referent group
HIV-infected	44.4 (22.4-68.7)	1.1 (0.4-3.2); p= 0.79
Solid Tumour	27.6 (18.8-38.4)	referent group
ST[4] localized	27.4 (17.2-40.4)	referent group
ST metastatic disease	28 (12.9-49.6)	Not Applicable
Haematological malignancy	47.6 (36.5-58.8)	**2.3 (1.2-4.5); p= 0.015**
HM[5] medium risk	10 (1.8-33.1)	referent group
HM high-risk	59.7 (46.5-71.7)	**11.9 (2.5-56.9); p= 0.002**

MUAC[1] = Mid-upper arm circumference, AMA[2] = Arm muscle are, TSFT[3] = Triceps skinfold thickness, ST[4] = Solid tumour, HM[5] = Haematological malignancy

Table 11: Factors Associated with All-Cause Mortality in Children Treated for Cancer

Parameter	Percentage of Death (95% CI)	Adjusted Odds Ratio (95% CI; p-value)
No Indwelling Catheters	44.4 (22.4-68.7)	referent group
Indwelling Catheters	38.4 (29.5-48.1)	0.8 (0.3-2.4); p= 0.69
No High-dose Corticosteroids	21.4 (12.9-33.2)	referent group
High-dose Corticosteroids	58.6 (44.9-71.1)	**4.9 (1.6-15.9); p= 0.007**
Treatment intensity[1] 1	23108 (13.9-35.5)	referent group
Treatment intensity 2	23.9 (13.1-39.1)	Not Applicable
Treatment intensity 3	63.8 (50.1-75.7)	**7.8 (1.9-31.1); p= 0.004** comparing 3 to 2
No MCSE[2]	30.6 (18.7-45.6)	referent group
At least one MCSE	40 (31.3-49.4)	1.2 (0.6-2.5); p= 0.67
No SSE[3]	28.9 (15.9-46.1)	referent group
At least one SSE	39.7 (31.448.6)	1.3 (0.6-2.9); p= 0.55
No Gram-positive bacteraemia	33.3 (22.0-46.8)	referent group
Gram-positive bacteraemia	39.5 (30.4-49.3)	0.8 (0.2-2.7); p= 0.66
No Gram-negative bacteraemia	32.5 (22.9-43.8)	referent group
Gram-negative bacteraemia	41.9 (31.5-52.9)	1.2 (0.5-2.8); p= 0.69
No Fungaemia	37.3 (29.2-46.1)	referent group
Fungaemia	37.1 (21.9-55.1)	0.9 (0.4-1.9); p= 0.74
No Pneumonia	27.7 (17.7-40.4)	referent group
Pneumonia	51.5 (38.9-63.9)	**2.4 (1.1-5.2); p= 0.021**
No Tuberculosis	33.6 (24.9-43.5)	referent group
Tuberculosis	66.7 (44.7-83.6)	**3.7 (1.4-9.8); p= 0.009**
No Urinary tract infection	35.1 (24.87-46.9)	referent group
Urinary tract infection	47.2 (33.5-61.2)	1.5 (0.7-3.2); p= 0.29
No Gastroenteritis	37.43 (27.6-48.2)	referent group
Gastroenteritis	46.2 (30.4-62.6)	1.1 (0.5-2.5); p= 0.86
No Mucositis	32.8 (21.9-45.8)	referent group

Mucositis	46.3 (34.2-58.8)	1.2 (0.5-2.6); p= 0.70
No Herpes stomatitis	34.8 (23.9-47.3)	referent group
Herpes stomatitis	45.2 (32.7-58.2)	1.1 (0.5-2.4); p= 0.80
No Profound[4] Neutropenia at onset of SE[5]	12.8 (5.3-26.4)	referent group
Profound Neutropenia at onset of SE	54.8 (43.6-65.5)	**6.7 (2.3-19.5); p<0.001**
No Prolonged[6] Neutropenia during SE	13.2 (4.9-28.9)	referent group
Prolonged Neutropenia during SE	50.5 (40.0-60.9)	**5.5 (1.8-16.9); p= 0.003**
No Profound Lymphopenia at onset of SE	14.3 (6.4-27.9)	referent group
Profound Lymphopenia at onset of SE	54.3 (42.9-65.3)	**5.4 (1.9-14.7); p= 0.001**
No Prolonged Lymphopenia during SE	18.4 (8.3-34.9)	referent group
Prolonged Lymphopenia during SE	48.4 (37.9-58.9)	**2.8 (1.0-7.7); p= 0.048**
No Profound Monocytopenia at onset of SE	14.8 (4.9-34.6)	referent group
Profound Monocytopenia at onset of SE	45.6 (35.9-55.7)	Not Applicable
No Prolonged Monocytopenia during SE	10.3 (2.7-28.5)	referent group
Prolonged Monocytopenia during SE	48.5 (38.5-58.6)	Not Applicable

Treatment intensity[1] = Standard intensity =1, Medium intensity = 2, High intensity = 3, MCSE[2] = microbiologically confirmed septic episode, SSE[3] = Suspected septic episode, Profound pancytopaenia[4] = absolute cell count < 100 cells/µL, SE[5] = Septic episode Prolonged[6] pancytopenia = absolute cell count < 1000 cells/µL for > 7 days

4.3. DISCUSSION

Many of our patients presented with advanced and aggressive disease, including 25% of children with ST presenting with Stage 4 disease, 61% of those with NHL had Stage 4 disease and 78% of children with ALL had high-risk disease. The presentation with such advanced disease, as well as the related high-intensity treatment regimen required to manage such children were independently associated with sepsis in our study. Our study was associated with high incidence (per 100 child years) of clinically suspected sepsis (overall = 103), which was higher among children with HM (141) than in those with ST (71). Similarly, the incidence of MCSE was also higher among children with HM (101) than in those with ST (46). In our study, 71% of all febrile illnesses were associated with a positive blood culture, which is higher than observed in other studies which ranged from 25% in paediatric studies (Hakim et al., 2009) to 52% in adult studies (Jagarlamudi et al., 2000).

In our study, children with HM were more likely to develop suspected sepsis and microbiologically confirmed sepsis. All 13 patients who died because of sepsis had HM. Age younger than ten years; metastatic ST, HM, and high intensity treatment were associated with an increased risk of MCSE in our cohort. Many studies from HICs (Sung et al., 2007, O'Connor et al., 2014) and LMICs (Bakhshi et al., 2008, Gupta et al., 2011a, Gavidia et al., 2012, Ghosh et al., 2012) have documented the frequency, aetiology, and outcome of sepsis in children with HM. However, very few have documented the same in children treated for ST (Kocak et al., 2002).

It is presumed that the cancer treatment for children with ST is less intensive than that for HM. Nevertheless, our study documented complicated septic episodes in children with ST associated with GPB, GNB, fungal and polymicrobial septic episodes. The reasons for this may be that 25% of the cohort with STs presented with Stage 4 disease and were treated with aggressive chemotherapy, advanced and complex surgery, and wide-field radiotherapy. The aggressive multi-modal therapy adds to the prolonged and profound myelosuppression and is associated with increased predisposition for infectious complications (Barson and Brady, 1987).

Over the past decades, the epidemiological spectrum of BSIs from febrile infections in patients treated for cancer has changed. During the 1960s, Gram-positive pathogens predominated. In the 1970s, 70% of bacterial isolates from cancer patients were Gram-negative pathogens, while in the 1980s and 1990s, probably due to the increased use of intravascular devices, fluoroquinolone prophylaxis, cotrimoxazole prophylaxis, and high-dose chemotherapy-induced mucositis, 70% of bacterial isolates from cancer patients with febrile illnesses are GPB (Zinner, 1999).

A report on 2142 adult cancer patients with febrile infections described 499 (23%) had bacteraemia (Klastersky et al., 2007). The relative frequencies of GPB, GNB, and polymicrobial bacteraemia were 57%, 34% and 10%, respectively. An Italian study described the frequency of GPB in paediatric cancer patients with febrile episodes which ranged from 57% to 80% (Castagnola et al., 2007). These findings were in contrast with other studies that showed that GNB remain the predominant pathogens, 61.8% in a Turkish study (Yilmaz et al., 2008), and 71% in an Indian study (Bothra et al., 2013) during febrile illnesses in children with cancer. Our study documented 51.1% of all microbiologically confirmed sepsis was due to GPB, 40.4% to GNB, and 8.5% to fungal pathogens.

Mikulska et al, in a literature review on bacteraemias in adult (29 studies) and paediatric cancer patients (16 studies), demonstrated the median GPB: GNB ratio was 58: 42, ranging from 86: 14 to 32: 68 in individual studies. The main pathogens were CONS (23%, range: 9-49%), *Enterobacteriaceae* (23%, range: 7-40%), *Streptococcus viridans* (13% range: 0-35%) and *Pseudomonas aeruginosa* (9%, range: 0-18 %) (Mikulska et al., 2014). Studies from El Salvador on febrile illnesses in children with cancer, documented 43% of all microbiologically confirmed infections were polymicrobial (Gupta et al., 2011a). In contrast, polymicrobial infections in patients treated for cancer in HICs are uncommon (Duncan et al., 2007). The presence of a polymicrobial infection has important implications for mana-gement as it could affect the selection of antimicrobial therapy. Many reports have focused attention only on BSIs caused by a single organism (monomicrobial BSIs) and have not included detailed information on polymicrobial BSIs or infections at other sites. Consequently, these data may provide an incomplete picture, because the overall spectrum of infection in patients with cancer may differ considerably from that associated with monomicrobial BSIs alone(Rolston et al., 2007).

Our study documented multiple SSE (overall 3.05 per patient), which was higher among children with HM (4.02 per child) than in ST cases (2.28 per child). The incidence of SSE among children with HM in our study was higher than reported from Morocco, where the rate of febrile illnesses (both microbiologically confirmed and culture negative) in patients with AML was a median of three episodes per patient, which was similar to that of HIC (Depasse et al., 2013). In one multicentre study in the United Kingdom (Dommett et al., 2009), children with AML developed an average of 3.03 FN per patient. In this same study, children with ALL developed on average 2.04 episodes per patient, which is lower than documented in our study.

We also reported a high frequency of fungaemia in both children with ST (18.4%) and HM (21.3%). In children treated for ST, a very low incidence of 0.4% has been documented between 1988 and 2000 by Ridola et al. (Ridola et al., 2004). Other studies have documented fungal infections ranging from 3.5% (El-Mahallawy et al., 2002) to 14.2% (Bothra et al., 2013).The reasons for the high incidence in our study may be multifactorial and could include that all children are treated with empiric broad-spectrum antibiotics at the onset of a septic episode, and prolonged and profound bone marrow suppression. Furthermore, high percentages of our subjects (35% of those with suspected sepsis, 41.9% of those with microbiologically confirmed sepsis were on corticosteroids, which are an essential part of treatment for all HM and brain tumours, and is an established risk factor for invasive fungal infections.

Although *Candida albicans* remains the most common pathogen in invasive fungal infections, non-*albicans* species are increasingly associated with invasive candidiasis (Tang et al., 2014). However, non-*albicans* species was uncommon in our patients. The increased frequency of *Candida* non-*albicans* in other settings could be attributable to the increased use of anti-fungal prophylaxis (Tang et al., 2014), which is not used routinely in our setting. This could also contribute to the high incidence of *Candida albicans* sepsis in our setting. There were only two cases of *Aspergillus* species associated fungaemia, which is not surprising as infection from this has been mainly identified in patients who undergo allogeneic stem cell transplantation (Thirumala et al., 2010). None of the patients in our study cohort had allogeneic stem cell transplantation.

There is a significant increase in the proportion of infections caused by multidrug-resistant bacteria, especially Gram-negative rods with extended-spectrum spectrum beta-lactamases (ESBLs), methicillin-resistant *Staphylococci* (MRSA), and vancomycin-resistant *Enterococc*i (VRE). In our study cohort, 89.3% of *Klebsiella* species, 69.2% of *Escherichia coli*, 40% of *Enterobacter* species, 10.3% of *Acinetobacter* species and 7.7% of *Pseudomonas* species were multi-resistant-negative pathogens. Thirteen percent of *Staphylococci* were MRSA, 65.5% of *Enterococcus faecalis* were VRE.

Enterobacter cloacae were resistant to 64% of fourth generation Cephalosporins, which is indicative of ampC β-lactamase hyper-production in combination with porin loss, which may confer resistance to Cephalosporins (Jacoby, 2009). *Escherichia coli* were resistant to 49-64% of the third generation Cephalosporins, which again indicates the presence of ESBL. *Klebsiella pneumoniae* was 100% sensitive to the Carbapenems. *Pseudomonas aeruginosa* isolates displayed greater susceptibility to the antimicrobial agents tested. None of the isolates in the study cohort was Carbapenemase-producing *Enterobacteriaceae*. In our study, *Acinetobacter baumanni* had a decreased sensitivity to most of the antimicrobials. The organism harbours multiple mechanisms of resistance such as loss of outer membrane porins, which reduces the permeability, efflux pump systems, and ampC β-lactamases (Bonomo and Szabo, 2006).

Our current policy on the empiric use of antimicrobials is outlined in Appendix 7. We identified a trend for increasing multi-drug GNB therefore changed our first-line empiric therapy to Carbapenems. Shortly after the study period, we experienced an outbreak of Carbapenemase-resistant *Enterobacteriaceae* in our unit with 80% mortality (unpublished data). We have now implemented stricter measures for the use of the Carbapenems. Epidemiological shifts occur periodically and have an effect on antimicrobial prophylaxis and empirical therapy. The global problem of increasing antimicrobial resistance is beginning to limit therapeutic options for the treatment of multi-resistant bacterial infections in patients with neutropenia as well. Unfortunately, the development of new drugs is not keeping pace with the development of resistance.

In our cohort of patients, age less than ten years, high-risk HM, high intensity treatment, high-dose corticosteroids, profound neutropenia and lymphopenia at the onset of sepsis, prolonged neutropenia and lymphopenia during a septic episode, and the presence of pneumonia and tuberculosis were associated with an increased risk of death.

We had a high percentage of patients with septic episodes presenting with pneumonia (24.5% HM, 17.1% ST). There are conflicting reports on the prevalence of pneumonia in children with FN. A Canadian study reports 5% of all febrile episodes in children with cancer were complicated by pneumonia (Renoult et al., 2004) and a study from El Salvador documented 12% of septic episodes had a clinical pneumonia and 29% had a radiological pneumonia. In a review of adult patients with FN a total of 15–30% of documented infections in FN are classified as pneumonia (Rolston et al., 2007).

There are many obstacles to the diagnosis of pneumonia in patients with FN. The patients are neutropenic, unable to produce adequate sputum specimens and because of neutropenia, chest radiological changes may be subtle. In addition, since many of these patients are critically ill, they are unable to tolerate diagnostic procedures such as broncho-alveolar lavage. In LMIC settings, radiology services, especially bedside services for critically ill patients, are limited. Both cancer and its treatment induce derangements of innate and adaptive immune function. Leucocyte depletion, dysregulated inflammation, and, impaired pathogen recognition contribute to cancer patients' susceptibility to LRTIs (Thirumala et al., 2010). Furthermore, recurrent healthcare encounters that are typical among cancer patients promote exposure to nosocomial pathogens. The impact of pneumonia on cancer populations is uniquely severe, accounting for more morbidity and mortality than any other infectious complication (Bakhshi et al., 2008, Thirumala et al., 2010, Ghosh et al., 2012). In our cohort, presentation with pneumonia was associated with an increased risk of death. Pneumonia has been described as a predictor of death in children with HM in both HIC (Lehrnbecher et al.,

2004) and LIC settings (Bakhshi et al., 2008, Gupta et al., 2011b, Ghosh et al., 2012). In our study, the diagnosis of pneumonia was made on clinical examination. The prevalence of pneumonia may be perhaps under-estimated by the lack of adequate radiology services. Early radiological detection of pneumonia may result in a change of antibiotic treatment and further optimization of therapy. Patients who develop acute respiratory failure may benefit from ventilator support in an ICU setting. None of the eight patients with pneumonia who required mechanical ventilation at the time of clinical deterioration in our study, were admitted to the ICU due to resource constrains. All eight patients subsequently demised. With earlier referral to ICU and improved management, the outcome may have been different in these subjects. In our study, pneumonia was an independent risk factor for mortality in the cohort who died of sepsis and in the overall cohort.

The majority of children in our cohort had severe malnutrition (76.9% MUAC < 5^{th} percentile and 56.8% AMA < 5^{th} percentile). However, there were no significant associations identified between malnutrition on admission and risk for infection or mortality in our study cohort. The prevalence of malnutrition in children diagnosed with WT at a South African institute was recorded at 31% (Wessels et al., 1999). In Malawi, paediatric oncology patients with acute malnutrition at diagnosis had a higher rate of neutropenia, prolonged neutropenia, profound neutropenia, febrile neutropenia, delays in treatment and death during treatment than those who were not malnourished (Israels et al., 2008). In a study from Guatemala, it was documented that > 50% of the children with cancer had some degree of malnutrition at diagnosis, reflecting both a possible delay in diagnosis and the general condition of the population living in Guatemala (Sala et al., 2008). Malnutrition was documented at 52% of children with ALL (kumar et al., 2000) and 56.8% of all cancers (Jain et al., 2003) in newly diagnosed Indian children. In Bangladesh, 53% of children are malnourished at diagnosis. These children had two to three times more culture and clinically proven infection, required a longer duration of induction and prolonged hospital stay (Hafiz and Mannan, 2008). In those children who had severe malnutrition after six months of treatment, the hazard of death was 2.4 fold compared to children with an adequate nutritional status or moderate malnutrition (Sala et al., 2012, Antillon et al., 2013). Malnourished children more often abandoned therapy and their event free survival was inferior to that of other children (Sala et al., 2012). In our study cohort, malnutrition had no significant relationship with infectious complications or death. The reasons for this are unclear.

The current study emphasises the importance of frequent assessment and analysis of type, frequency, severity, and outcome of infectious complications to detect changing epidemiological patterns. There is a need for improved diagnostic tests to detect infections early. It also stresses the need to treat fungal infections and TB early, as they were a major cause of sepsis in our patients. These findings emphasise the need to aggressively investigate and treat children with cancer for TB, pneumonia and other septic complications.

Unquantified factors, which could have contributed to the infectious complications in our study, include overcrowding, lack of adequate medical, nursing and cleaning staff, lack of isolation facilities hence patients are constantly in an environment with a high microbial load. Additionally, patients are not discharged home frequently, as transport is expensive and if these patients were to be discharged home, transport back to the hospital may not be timeous, and chemotherapy-induced febrile illnesses are an oncological emergency with a high mortality. These may be some of the multitude of reasons for the high number of suspected and microbiologically confirmed septic episodes.

5. TUBERCULOSIS IN CHILDREN WITH CANCER

5.2. CLINICAL CHARACTERISTICS OF TUBERCULOSIS IN CHILDREN TREATED FOR CANCER

Mycobacterium tuberculosis infection, as measured by a positive response to the TST was documented in five of 169 (2.99%) children, all of whom were empirically treated with the full course of anti-TB treatment. None of these five children was subsequently diagnosed with active TB disease during the course of their treatment. The T-SPOT.TB test yielded non-reactive results in the first 100 children enrolled in the study, and none of the tests had an indeterminate result. Consequently, further testing of subsequent children enrolled into the study using the T-SPOT.TB test was terminated.

Twenty-four (14.2%) of the 169 children enrolled into the study were diagnosed with TB during the course of their cancer treatment, 7.5% for HM compared with ST (3.1%; p=0.0.36). The incidence ratio of developing TB for HM compared to ST was 2.39 fold (95% CI: 0.96-6.45); Table 12. The diagnosis of TB was microbiologically confirmed among 45.8% (11/24) of the TB cases, all of which were based on a positive culture for *Mycobacterium tuberculosis*, whereas the remaining 13 (54.2%) of cases were diagnosed based on clinical suspicion. Seven (43.8%) of 16 TB cases among children with HM and 4 (50%) of the eight TB cases in children with ST were based on culture-confirmed disease; Table 12.

The incidence (per 100 child years) of TB was higher among children with HM (6.8) than those with ST, (2.9; p=0.043). Overall, the proportion of HIV-infected children with a malignancy who developed TB was (4/18; 22.2%) and was (20/151; 13.2%) among HIV-uninfected children. Among children with HM, 23.1% (3/13) of the HIV-infected children were diagnosed with TB compared to 20% (1/5) of HIV-uninfected children; Table 13.

Children who developed TB had a higher number of high-dose corticosteroids courses (397.4 per 100 child years) compared to those who did not develop TB (40.7 courses per 100 child-years; p<0.001; Table 14). In addition, children who developed TB were more likely to have septic episodes (rate per 100 child years) associated with profound neutropenia (319.5. vs 46.6; $p<0.001$), profound lympho-penia (264.9 vs 41; $p<0.001$) and profound monocytopenia (389.6 vs 63.7; $p<0.001$) prior to the diagnosis of TB than those who did not develop TB; Table 14. Similarly, children who developed TB had more septic episodes with prolonged neutropenia (350.6 vs 51.1; $p<0.001$) and prolonged lymphopenia (335.1 vs 59.2; $p<0.001$), and prolonged monocytopenia (444.2 vs 70.7; $p<0.001$) prior to developing TB than children who did not develop TB.

Table 12: Malignant Diseases Associated with Proven and Probable Tuberculosis

Parameter	Overall N=24	Proven TB N=11	Probable TB N=13	*p-value
Haematological Malignancy	16/24 (66.7%)	7(63.6%)	9(69.2%)	>0.99
ALL[2]	9/16 (56.3%)	4(57.1%)	5(55.6%)	>0.99
AML[3]	3/16 (18.8%)	1(14.3%)	2(22.2%)	>0.99
NHL[4]	4/16 (25.0%)	2(28.6%)	2(22.2%)	>0.99
Solid Tumours	8/24 (33.3%)	4(36.4%)	4(30.8%)	>0.99
WT[5]	4/8 (50.0%)	1(25.0%)	3(75.0%)	0.49
RMS[6]	1/8 (12.5%)	1(25.0%)	0(0.0%)	>0.99
BT[7]	1/8 (12.5%)	1(25.0%)	0(0.0%)	>0.99
ACC[8]	1/8 (12.5%)	0(0.0%)	1(25.0%)	>0.99
KS[9]	1/8 (12.5%)	1(25.0%)	0(0.0%)	>0.99

N= number of patients, TB[1] = Tuberculosis, ALL[2] = Acute Lymphoblastic Leukaemia, AML[3] = Acute Myeloid Leukaemia, NHL[4] = Non-Hodgkin's Lymphoma, WT[5] = Wilm's Tumour, RMS[6] = Rhabdomyosarcoma, BT[7] = Brain tumour, ACC[8] = Adrenocorticoid carcinoma, KS[9]=Kaposi's sarcoma. NA=Non-applicable, *p= proven vs probable TB

Table 13: Clinical Characteristics of Tuberculosis in Children Treated for Cancer

Parameter	Overall N=169	Haematological Malignancy N=82	Solid Tumour N=87	p-value*
Number of patients with Tuberculosis	24 (14.2%)	16 (19.5%)	8 (9.2%)	
Person time (years)	475	231	262	
Incidence of Tuberculosis per 100 child years (95% Confidence Interval)	4.7 (2.8-6.5)	6.8 (3.5-10.2)	2.9 (0.9-4.8)	**0.043**
Mean age at presentation of cancer in months in children with TB (SD[1])	76.7 (46.9)	71.3 (47.19)	71.5 (49.5)	0.72
HIV-infection prevalence in TB cases	4 (16.7%)	3 (18.8%)	1 (12.5%)	0.45
Median age at TB[2] presentation in months (IQR[3])	75.5 (43.8-122.5)	84.5 (48.3-122.5)	65.0 (42.3-101.3)	0.83
Mean time from presentation to TB diagnosis- months (SD)	5.4 (6.9)	4.0 (6.0)	8.3 (8.1)	0.22

N= number of patients, SD[1] = Standard deviation, TB[2] = Tuberculosis, IQR[3] =interquartile range, *p = Haematological Malignancy vs Solid Tumour

5.3. Predictors of Tuberculosis in Children Treated for Cancer

There was no identifiable association between age group, HIV status, tumour type, stage of tumour, treatment intensity or nutritional status at enrolment and the risk of being diagnosed with TB; Table 15.

5.4. Death Associated with Tuberculosis in Children with Cancer

There were four (16.7%) deaths among the 24 children who were diagnosed with TB, including two each with ALL and NHL; Table 16. Two of those who died were HIV-infected (both had NHL). All four had microbiologically confirmed TB. Furthermore, three of the four children who died had concurrent bacteraemia (two had multi-drug resistant Gram-negative bacteraemia) and all four had concurrent respiratory viral coinfections.

Table 14: White Cell Differential and High-dose Corticosteroid Exposure Prior to the Diagnosis of Tuberculosis

Parameter	Tuberculosis N[6]=13	No Tuberculosis N=357	p-value*
Number** of cycles of high-dose Corticosteroids prior to TB diagnosis (95% CI [1])	397.4 (288.3-506.5); n[7]=51	40.7 (34.1-47.3); n=145	**<0.001**
Number of SE[2] with prolonged neutropenia prior to TB[3] diagnosis (95%CI)	350.6 (248.2-453.1); n=45	51.1 (43.6-58.5); n=182	**<0.001**
Number of SE with profound[4] neutropenia prior to TB diagnosis (95%CI)	319.5 (221.7-417.3); n=41	46.6 (39.5-53.7); n=166	**<0.001**
Number of SE prolonged[5] lymphopenia prior to TB diagnosis (95%CI)	335.1 (234.9-435.2); n=43	59.2 (51.2-67.2); n=211	**<0.001**
Number of SE with profound lymphopenia prior to TB diagnosis (95%CI)I	264.9 (175.9-354); n=34	41 (34.3-47.6); n=146	**<0.001**
Number of SE with prolonged monocytopenia prior to TB diagnosis (95%CI)	444.2 (328.8-559.5); n=57	70.7 (62-79.4); n=252	**<0.001**
Number of SE with profound monocytopenia prior to TB diagnosis (95%CI)	389.6 (281.6-497.6); n=50	63.7 (55.4-72); n=227	**<0.001**

CI[1] = Confidence interval, SE[2] = Standard deviation, TB[3] =Tuberculosis, Profound[4] = Absolute cell count < 100 cells/µL, Prolonged[5] = Absolute cell count < 1000 cells/µL for > 7 days, N[6] = Number of patients, n[7] = number of events, **per 100 child years

Table 15: Predictors of Tuberculosis in Children Treated for Cancer

Parameter	Percentage of Tuberculosis (95% CI)	Adjusted Odds Ratio (95% CI); p-value
Age (years)		
1-5	15.17 (8.1-25.8)	1.3 0.4-3.9); p= 0.64
>5-10	14 (6.3-27.4)	1.2 (0.4-4.1); p= 0.74
>10	13.3 (5.5-27.5)	referent group
Female	16.4 (9.1-27.45)	referent group
Male	12.5 (6.9-21.2)	0.6 (0.2-1.5); p= 0.29
MUAC[1]>5th percentile	11.1 (3.6-27)	referent group
MUAC<5th percentile	15.4 (9.9-23)	1.5 (0.5-4.8); p= 0.51
AMA[2]>5th percentile	12.9 (6.4-23.5)	referent group
AMA<5th percentile	15.6 (9.3-24.8)	1.2 (0.5-2.9); p= 0.71
TSFT[3]>5th percentile	15.8 (9.6-24.8)	referent group
TSFT<5th percentile	12.3 (5.8-23.4)	0.7 (0.3-1.9); p= 0.53
HIV-uninfected	13.3 (8.5-19.9)	referent group
HIV-infected	22.2 (7.4-48.1)	1.7 (0.5-6.2); p= 0.42
Solid Tumour	9.2 (4.4-17.8)	referent group
ST[4] local disease	9.7 (4-2-5.4)	referent group
ST metastatic disease	8 (1.4-27.5)	0.7 (0.1-3.7); p= 0.65
Haematological malignancy	19.5 (11.9-30.0)	1.8 (0.7-4.7); p= 0.2
HM[5] medium risk	5 (0.3-26.9)	referent group
HM high-risk	24.2 (14.6-37.0)	5.4 (0.7-44.2); p= 0.12
Treatment intensity[6] 1	7.7 (2.9-17.8)	referent group
Treatment intensity 2	8.7 (2.8-21.7)	1.1 (0.3-4.7); p= 0.85
Treatment intensity 3	25.9 (15.7-39.3)	5.2 (0.8-32.9); p= 0.08
No MCSE[7]	4.1 (0.71-15.1)	referent group
At least one MCSE	18. (12.1-26.7)	4.4 (0.9-20); p= 0.055
No SSE[8]	0 (0-11.4)	referent group
At least one SSE	18.3 (12.3-26.2)	Not Applicable

MUAC[1] = Mid-upper arm circumference, AMA[2] = Arm muscle area, TSFT[3] = triceps skinfold thickness, ST[4] = solid tumour, HM[5] = haematological malignancy, treatment intensity[6] 1= Standard intensity, 2 = medium intensity, 3 = high intensity, MCSE[7] = microbiologically confirmed septic episode, SSE[8] = Suspected septic episode

Table 16: Death Associated with Tuberculosis in Children with Cancer

Pt	Sex	Age (months)	Disease	Time from TB diagnosis to Death	HIV	Blood culture	Sputum	Virus	Clinical Syndrome
1	M	125	ALL[1]	6 weeks	Neg	*Bacillus, Klebsiella pneumoniae*	MTB[3]	CV[5]OC43, CVHUK1	Pneumonia, Herpes stomatitis, Proven TB
2	F	100	NHL[2]	4 weeks	Pos	Nil	MTB	FluA[6], HRV[7], CVOC43, CVNL63	Pneumonia, Herpes stomatitis, Mucositis, Proven TB
3	F	98	ALL	5 weeks	Neg	ESBL[4] *K. pneumoniae*	MTB	CVNL63, KIPyV[8]	Pneumonia, Gastroenteritis, Proven TB
4	M	23	NHL	3 weeks	Pos	ESBL *E coli*	MTB	HRV, CVNL63	Pneumonia, Herpes stomatitis, Proven TB, Mucositis

ALL[1] = Acute lymphoblastic leukaemia, NHL[2] = Non=Hodgkin’s lymphoma, MTB[3] = Mycobacterium tuberculosis, ESBL[4] = Extended-spectrum β-lactamase inhibitor, CV[5]= human Coronavirus, FluA [6] = Influenza A, HRV[7]= human Rhinovirus, PyV[8] = human Polyomavirus

5.5. DISCUSSION

The incidence for TB in our study cohort was 4.7 per 100 child years (95% CI: 2.8-6.5), 6.8 (95% CI; 3.5-10.2) in children with HM, and 2.9 (95% CI: 0.9-4.8) in children with ST. However, almost 50% of the patients in our study were culture positive, which is very high for children. Stefan et al reported an incidence of 9.14 per 100 child years among children with cancer, in the Western Cape, South Africa (1999 to 2005). Only children with a positive culture for MTB, positive sputum smear or clinical and radiological signs of active TB were included in the study. Stefan et al estimated at that time, the risk of developing TB in children with cancer was 22.4 times higher than in the general population which was estimated at 0.47 per 100 child years, for the study period 1999 to 2005 (Marais et al., 2006a, Stefan et al., 2008b). The lower incidence in our study may be due to a general decline in the incidence of TB since the mid-2000, including among children <15 years of age (Nanoo et al., 2015).

The study by Stefan et al. documented 53% of TB occurred in children with HM, 10.5% were HIV-infected 63.2% were males and the time from diagnosis of cancer to diagnosing TB was 7.6 months (Stefan et al., 2008a) . In our study, 66.7% of the cohort with TB had a HM, 16.7% were HIV-infected, and the time from diagnosis of cancer to TB was shorter at 5.5 months (4.19 months for HM, and 8.12 months for children with ST).

The diagnosis of TB is often delayed among children, especially those who are immune-compromised, due to both a low index of suspicion and unavailability of diagnostic tests (Narasimhan et al., 2013). In addition, young children and immunocompromised patients are more likely to have a negative TST, and less likely to have positive sputum staining for AFB (Klossek et al., 2004). Broncho-alveolar lavage is reported to be an effective and well-tolerated technique for the microbiological diagnosis of pneumonia, including TB, in adult patients with HM (Cordani et al., 2008), but in settings such as ours this technique is not readily available. In addition, many children with cancer may have an increased risk for bleeding, due to complications of the disease or treatment, hence are not suitable candidates for invasive procedures such as broncho-alveolar lavage. In our study cohort, all children had a T-SPOT.TB test on enrolment into the study, however, the first 100 patients yielded a negative result, and the T-SPOT.TB test was abandoned. Very low sensitivity and specificity were found on analysis of IGRA tests in studies in immunocompromised children. The meta-analysis estimate for sensitivity was only 0.54 for T-SPOT.TB test, and 0.47 for QFT-G-IT, confirming that IGRA results should be used with caution in immunocompromised children. In LMICs and in immunocompromised children, IGRAs' performance is equivalent or inferior to TST (Sollai et al., 2014). TST is a suboptimal test for screening for TB. The population infection rate is 3% year-on-year; therefore, in our study cohort who presented with a median age of 6.4 years, the expected TST positivity rate would have been 19.2% instead of our result of 2.9%. We have to be cautious when TB is excluded based on a negative TST result.

The balance between the tubercle bacillus and the immune system influences the development of active TB from latent TB infection. Patients with HM have an underlying immunological deficiency that increases the risk for infections (Pagano et al., 2012). Alteration in the immune response of patients with cancer, leads to an impaired immune response that promotes the progression from latent TB infection to active TB (Al-Anazi et al., 2007).

In low-burden TB areas, the risk of active TB disease is also increased. In the USA, it was documented that adult patients with HM who had been treated with haemopoietic stem cell transplantation had 40 times the risk of developing active TB disease than the general population (Kamboj and Sepkowitz, 2006). Adults Indian patients with leukaemia, especially AML have a 23 times increased risk of developing active TB disease(Mishra et al., 2006).

There was no identifiable association between age group, HIV status, and tumour type, stage of tumour, treatment intensity, or nutritional status at enrolment and the risk of being diagnosed with TB. The most likely explanation for this is the small number of patients with cancer who developed TB

In our children diagnosed with TB, we documented all patients had septic episodes with prolonged and profound pancytopenia prior to the diagnosis of TB. Other studies did not document the haematological parameters in patients with cancer and TB except for one study from Taiwan. Chen et al reported that tuberculosis in adult AML patients was associated with significant febrile neutropenia compared to non-AML patients. The risk of acquiring TB disease correlated partly with the patient's absolute neutrophil count (Chen et al., 2012).

Recent research on neutrophil peptides has documented that neutrophils are bactericidal against TB, which suggests that neutrophils play an important role in the defence against tuberculosis via the innate immune system. In an adult non-cancer TB cohort, the risk of TB disease was inversely and independently associated with peripheral blood neutrophil counts on enrolment into the study, in patients diagnosed with pulmonary TB (Martineau et al., 2007). This result was consistent with our study supporting the important role of neutrophils in the defence against TB infection.

In our cohort of children with TB, malnutrition was highly prevalent on enrolment into the study; however, the presence of malnutrition did not predict an increased risk for TB in our cohort. We did, however, by using a logistic regression model establish that children who developed TB were treated with more courses of high-dose corticosteroids than those who did not develop TB (109 vs 10 courses per 100 child years), indicating this to be risk factor for developing TB. This was similar to a report by Silva et al, who too reported that pre-treatment with corticosteroids was a risk factor for the development of TB disease in cancer patients (Silva et al., 2005). In addition, a Saudi Arabian study documented that 61% of adult patients with cancer and TB had received corticosteroids prior to the diagnosis of TB (Al-Anazi et al., 2007). The increased risk of TB in patients receiving high-dose corticosteroids could be due to its immunosuppressive effects on cell-mediated immunity. High-dose corticosteroid treatment has also been linked to increased disease severity of TB at presentation, increased risk for miliary TB, and higher case fatality rates from TB (Lancioni et al., 2009) .

Thirteen patients in our study patients died because of sepsis, including four (36.4 %) of 11 children who had microbiologically confirmed TB. Furthermore, three of the four children who died had concurrent bacteraemia (two had multi-drug resistant Gram-negative bacteraemia) and all four had concurrent respiratory viral co-infections. The cohort of patients, who died, in addition to MTB, had co-infections with bacteria and respiratory viruses. This suggests a complex disease process at the time of death. Post-mortem studies were not conducted in these patients; hence, it is difficult to delineate the role of the pathogens identified as the cause of death. Some of the viruses may have been co-incidentally detected in the airway at the time of death. Other studies, in adult cancer patients with TB, have recorded even higher mortality figures, including that by Silva et al (62%) (Silva et al., 2005). Also, a study from the USA in adult cancer patients with TB, documented a 48% mortality in the 1970s (Kaplan et al., 1974). There are no recent studies documenting mortality from TB in children with cancer, and none of the children with cancer and TB in the study from the Western Cape, died (Stefan et al., 2008b).

The five cases that had a positive TST on admission were treated with a full course of empirical TB treatment, and none subsequently developed TB. This indicates that despite the low yield positivity (3%), it remains imperative to try to establish a diagnosis of latent TB infection and consider preventive therapy that could avoid the progression from a latent state to active TB. The TST was not a useful test in our study population. High rates of false negative results have been reported in HIV-infected children. In a study in Cape Town, among almost 300 children with TB, those with HIV-infection were less likely to have a positive TST than those who were HIV negative (36% versus 59%) (Moyo et al., 2010). In a further study in South Africa among HIV-infected children with culture-confirmed TB, only 56% had a positive TST (Hesseling et al., 2005).

In our study, we did not utilize any of the nucleic acid amplification tests that are available for the rapid diagnosis of TB. In 2011, World Health Organization (WHO) endorsed the Gene Xpert (Gen Xpert MTB/RIF®) test for the diagnosis of TB but there were no specific recommendations for its use in children because of limited data. In October 2013, the WHO recommended that Gene Xpert should be used instead of the conventional microscopy, for the initial diagnosis in children suspected of having MDR TB or HIV-associated TB. It was also recommended that Gene Xpert be used rather than conventional microscopy and culture as the initial test in all children suspected of having TB. The test is simple to use, has a high sensitivity and it provides rapid diagnosis of TB. Additionally, this method, detects an eventual resistance to rifampicin in less than two hours after obtaining the sample (WHO, 2013).

Subsequently, a study from Vietnam confirmed that Gene Xpert is a suitable, rapid and specific method for the diagnosis of childhood TB with approximately twice the sensitivity of smear microscopy (Giang et al., 2015). In future, Gene Xpert is a test that should be utilized together with TST and IGRA to increase the yield in children with cancer and TB.

The diagnosis of TB is often challenging, because it is not easy to obtain microbiological confirmation in such cases. However, in children it is recommended starting empirical anti-tuberculosis treatment, if TB is suspected. The children in the study cohort did not receive Isoniazid prophylaxis. In a study from Kenya it is documented that Isoniazid prophylaxis reduced the risk of developing tuberculosis by 59% among children aged 15 years or younger (Ayieko et al., 2014).

In a high-burden setting for TB, it is an important disease in children with cancer. Extreme care has to be taken to exclude latent TB and a high suspicion has to be maintained for active TB. In our cohort, 2.9% of TST was positive, none of the IGRA tests was positive but 50% of the cohort with TB had microbiologically confirmed TB. In addition, four children who died had microbiologically confirmed TB and co-infections with multiple bacteria (including multi-drug resistant bacterial pathogens) and respiratory viruses. However, the significance of the pathogens in relation to death is unknown. It may suggest a complex disease pathogenesis. The recommendation for children with cancer in a high-burden disease setting, especially those with HM, stem cell transplant and those with dual pathologies of cancer and HIV-infection, would be active exclusion of latent TB, high clinical suspicion for TB and that all patients be initiated on Isoniazid prophylaxis

6. Respiratory Viral Infections in Children Treated for Cancer

6.2. Results

6.2.1. Demographics of Respiratory Viral Associated Infections and Co-infections with Bacteria and Fungi in Children treated for Cancer

Of the 528 suspected septic episodes (SSE), 211 (37.3%) were associated with the identification of a respiratory virus. The identification of at least one respiratory virus per SSE episode was more common among children with haematological malignancies (HM; 44.5%) compared to those with solid tumours (ST; 32.3%; p=0.007); Table 17. One hundred and forty-two (68.3%) of respiratory virus associated septic episodes (SE) had one virus identified, including 98 (66.7%) of the episodes among HM and 44 (68.8%) in children with ST (p=0.77). Of the remainder, respiratory virus associated SEs, 22.2% had two respiratory viruses identified and 10.43% had > two viruses identified concurrently; data not shown. There was no association between the number of viruses detected and tumour type (p=0.77; data not shown). Human rhinovirus was the most frequent virus identified in combination with other viruses, including eight cases with concurrent Adenovirus, seven with CV NL63 and six each with RSV and KIPyV; Table 18.

Table 17: Demographics of Respiratory Viral Associated Infections and Co-infections with Bacteria and Fungi in Children treated for Cancer

Characteristic	Overall N=169	Haematological Malignancy N=82	Solid Tumour N=87	p-value*
Total number of SSE[1]	528	330	198	
Number of SSE associated with respiratory virus	211 (37.3%)	147(44.5%)	64(32.3%)	**0.007**
Number of SSE cases with respiratory virus identified and concurrent bacteraemia/fungaemia	157 (27.4%)	112 (33.9%)	45 (22.7%)	**0.013**
Number of SSE with 1 respiratory virus	142/211 (67.30%)	98/147 (66.67%)	44/64 (68.75%)	0.77
Number of SSE with 2 respiratory viruses	47/211 (22.27%)	37/147 (25.17%)	10/64 (15.62%)	0.12
Number of SSE with > 2 respiratory viruses	22/211 (10.43%)	12/147 (8.16%)	10/64 (15.62%)	0.28
Number of SSE with > 3 respiratory viruses	69/211 (32.70%)	49/14 (33.33%)	20/64 (31.25%)	0.77
Number of SE with GPB[4] and respiratory virus	86 (54.8%)	47 (15.6%)	39 (22.9%)	0.13
Number of SE with GNB[5] and respiratory virus	71 (45.2%)	46 (15.2%)	25 (13.6%)	0.53
Number of SE with Fungus and respiratory virus	26 (16.6%)	15(4.6%)	11 (5.7%)	0.56

N= number of patients, SSE[1] = Suspected Septic Episode, BC[2] = Blood Culture positive for bacteria or fungi, SE[3] = Septic Episode, GPB[4] = Gram-positive Bacteria, GNB[5] = Gram-negative Bacteria, *p-value: comparing Haematological Malignancy to Solid Tumour cases

Table 18: Contribution of Respiratory Viruses in Children with Cancer as Single (diagonal) or Coinfection (matrix) Infection

	ADV n=21 (6.9%)	FluA n=17 (5.3%)	FluB n=7 (2.2%)	RSV n=21 (6.6%)	HMPV n=11 (3.5%)	HRV n=9 (30.2%)	PIV1 n=11 (3.5%)	PIV2 n=7 (2.2%)	PIV3 n=6 (1.9%)	HBoV n=11 (3.5%)	CV22E n=1 (0.3%)	CVO43 n=20 (6.3%)	CVNL63 n=22 (6.9%)	CVHUK1 n=26 (8.2%)	WUPV n=1 (3.8%)	KIPyV n=29 (9.1%)
ADV[1]	7															
Flu[2] A	0	6														
Flu B	0	1	3													
RSV[3]	1	0	1	10												
HMPV[4]	0	3	4	1	4											
HRV[5]	8	3	1	6	2	55										
PIV[6] 1	0	0	1	1	1	4	3									
PIV 2	0	0	1	1	1	2	5	1								
PIV 3	0	1	0	0	0	2	2	2	2							
HBoV[7]	2	0	0	1	0	3	0	0	1	5						
CV[8] 229E	1	0	0	0	0	0	0	0	0	0	0					
CV OC43	2	2	0	0	0	6	0	0	0	1	0	9				
CV NL63	0	2	0	3	0	7	0	0	0	1	0	3	10			
CV HUK1	0	3	2	3	3	5	0	0	0	1	0	3	3	12		
WUPyV[9]	3	0	1	1	1	4	1	1	0	1	0	0	0	0	3	
KIPyV[10]	1	2	0	0	0	6	0	1	1	2	0	0	3	0	2	13

ADV[1]= Adenovirus, Flu[2] = Influenza, RSV[3] = Respiratory syncytial virus, HMPV[4] = human Metapneumovirus, HRV[5] = human Rhinovirus, PIV[6] = Parainfluenza virus, HBoV[7] = human Bocavirus, CV[8] = Coronavirus, WUPyV[9] =WU human Polyomavirus, KIPyV[10]=KI human Polyomavirus

Table 19: Respiratory Virus Associated Infections in Children Treated for Cancer

Incidence of viral associated Suspected Septic Episodes	Overall N =169	Haematological Malignancy N=82	Solid Tumour N=87	p-value*
Mean number of viral infection per patient (SD[1])	1.9	2.6(3.5)	1.1(2.1)	0.09
Person time of follow-up- years	514	234	280	
Viral associated SSE** (95%CI)	61.2 (54.4-68); n=314	92.4 (80.1-104.8); n=216	35.1 (28.1-42); n=98	**<0.001**
Human Rhinovirus (95% CI)	18.7 (15-22.5); n=96	29.1 (22.2-36); n=68	10 (6.3-13.7); n=28	**<0.001**
Coronavirus (95% CI[2])	13.4 (10.3-16.6); n=69	19.3 (13.6-24.9); n=45	8.6 (5.2-12); n=24	**0.002**
CV[3] 229E (95% CI)	0.2 (-0.2-0.6); n=1	0.4 (-0.4-1.3); n=1	0 (0-0); n=0	0.41
CV OC43 (95% CI)	3.9 (2.2-5.6); n=20	4.3 (1.6-6.9); n=10	3.6 (1.4-5.8); n=10	0.69
CV NL63 (95% CI)	4.3 (2.5-6.1); n=22	5.6 (2.5-8.6); n=13	3.2 (1.1-5.3); n=9	0.21
CV HUK1 (95% CI)	5.1 (3.1-7); n=26	9 (5.1-12.8); n=21	1.8 (0.2-3.4); n=5	**<0.001**
Polyomavirus (95% CI)	7.2 (4.9-9.5); n=37	12 (7.5-16.4); n=28	3.2 (1.1-5.3); n=9	**<0.001**
WUPyV[4] (95% CI)	2.3 (1-3.7); n=12	3.9 (1.3-6.4); n=9	1.1 (-0.1-2.3); n=3	**0.044**
KIPyV (95% CI)	4.9 (3-6.8); n=25	8.1 (4.5-11.8); n=19	2.1 (0.4-3.9); n=6	**0.003**
Parainfluenza virus (95% CI)	4.7 (2.8-6.5); n=24	6.8 (3.5-10.2); n=16	2.9 (0.9-4.8); n=8	**0.043**

PIV1[5] (95% CI)	2.1 (0.9-3.4); n=11	2.6 (0.5-4.6); n=6	1.8 (0.2-3.4); n=5	0.56
PIV2 (95% CI)	1.4 (0.4-2.4); n=7	2.6 (0.5-4.6); n=6	0.4 (-0.3-1.1); n=1	**0.035**
PIV3 (95% CI)	1.2 (0.2-2.1); n=6	1.7 (0-3.4); n=4	0.7 (-0.3-1.7); n=2	0.30
Respiratory Syncytial virus (95% CI)	4.1 (2.3-5.8); n=21	5.1 (2.2-8); n=12	3.2 (1.1-5.3); n=9	0.29
Adenovirus (95% CI)	4.1 (2.3-5.8); n=21	5.6 (2.5-8.6); n=13	2.9 (0.9-4.8); n=8	0.14
Influenza A virus (95% CI)	3.3 (1.7-4.9); n=17	6 (2.9-9.1); n=14	1.1 (-0.1-2.3); n=3	**0.003**
Influenza B virus (95% CI)	1.4 (0.4-2.4); n=7	1.7 (0-3.4); n=4	1.1 (-0.1-2.3); n=3	0.54
Human Metapneumovirus (95% CI)	2.1 (0.9-3.4); n=11	3.9 (1.3-6.4); n=9	0.7 (-0.3-1.7); n=2	**0.017**
Bocavirus per 100 child years (95% CI)	2.1 (0.9-3.4); n=11	3 (0.8-5.2); n=7	1.4 (0-2.8); n=4	0.24

N=number of patients, SD[1] = Standard deviation, CI[2] = Confidence interval, CV[3] = Coronavirus, PyV[4] = Polyomavirus, PIV[5] = Parainfluenza virus, *p = Haematological Malignancy vs Solid Tumour, Incidence**per 100 child years

6.2.2. Respiratory Virus Associated Septic Episodes in Children with Cancer

The overall incidence (per 100 child years) of respiratory virus associated SSEs was 61.2, which was higher in children with HM (92.4) than those with ST were (35.1; p<0.001); Table 19. The most frequently identified viruses were human Rhinovirus (n=96; 30.6%), human Coronavirus (n=69; 22%) and human Polyomavirus (n=37; 11.8%). In addition, 6.7% of SSE episodes were associated with RSV and Adenovirus each, and 5.4% with Influenza A virus.

The overall incidence (per 100 child years) of human Rhinovirus was 18.7, (29.1 HM vs 10 ST; p<0.001). Similarly there was higher incidence of human Coronavirus (19.3 vs 8.6; p=0.002), human Polyomavirus (12 vs 3.2 p<0.001), Influenza A virus (six vs 1.1; p=0.003), and human Metapneumovirus (3.9 vs 0.7; p=0.017) associated SSE episodes among children with HM than those with ST; Table 19.

6.2.3. Septic Episodes with Respiratory Virus and Bacterial/Fungal Co-infections in Children Treated for Cancer

Overall, 157(27.4%) septic episodes had co-infections with respiratory viruses and bacteria/fungi, 112(33.9%) in those with HM, and 45 (22.7%) in those with ST (p=0.013). The most frequently associated viruses with bacterial/fungal co-infections were human Rhinovirus (32.9%, n=64), human Coronavirus (23.2%, n=45), and human Polyomavirus (11.9%, n=23); Table 19.

The overall incidence (per 100 child years) of HRV with bacterial/fungal co-infections was 12.5, 20.1 in those with HM, and 6.1 in those with ST (p<0.001). Similarly, human Coronavirus (13.3 vs 5; p=0.003), human Polyomavirus (7.7 vs 1.8; p=0.002), Influenza A virus (3.9 vs 0.7; p=0.017), human Metapneumovirus was more frequently associated with bacterial/ fungal co-infections in children.

Human Rhinovirus was identified as a sole virus in 32 (52.5%) of SEs and as part of a bacterial/fungal co-infection in 64 (42.7%) of SEs. KIPyV was a sole virus in 10 (16.4%) and as part of a co-infection in 15 (10%) SEs, CV HUK1 was a sole virus in eight (13.1%) and 18 (12%) of SEs with bacterial/fungal co-infections, and RSV was a sole virus in nine (14.8%) and 12 (8%) of coinfections. The overall incidence (per 100 child years) of human Rhinovirus co-infection with bacteraemia/fungaemia was 13, which was greater in children with HM (21) that those with ST (7; $p<0.001$); Table 20. Similarly there was a higher incidence of respiratory virus associated SSE with concurrent bacteraemia/fungaemia with human Coronavirus (14 vs 5; $p=0.002$), human Polyomavirus (8 vs 2; $p=0.002$), Influenza A (4 vs 1; $p=0.017$), and human Metapneumovirus (3 vs 0; $p=0.035$) among those with HM than ST; Table 20. The most frequently associated respiratory viruses with bacteraemia/fungaemia co-infections were human Rhinovirus (n=69; 30.8%), human Coronavirus (n=48; 21.4%), and human Polyomavirus (n=23; 10.3%).

Table 20: Mixed Bacterial and or Fungal and Respiratory Viral Co-infections in Children Treated for Cancer

Parameter	Overall N=169	Haematological Malignancy N=82	Solid Tumour N=87	P*-value
Number of SSE cases with respiratory virus identified and concurrent bacteraemia/ fungaemia	157 (27.4%)	112 (33.9%)	45 (22.7%)	**0.013**
Bacterial/Fungal Co-infection with human Rhinovirus** (95% CI[1])	12.5 (9.4-15.5); n=64	20.1 (14.4-25.9); n=47	6.1 (3.2-9); n=17	**<0.001**
Bacterial/Fungal Co-infection with human Coronavirus (95% CI)	8.8 (6.2-11.3); n=45	13.3 (8.6-17.9); n=31	5 (2.4-7.6); n=14	**0.003**
Bacterial/Fungal Co-infection with CV[2] 229 E (95% CI)	0 (0-0); n=0	0 (0-0); n=0	0 (0-0); n=0	0.93
Bacterial/Fungal Co-infection with CV OC 43 (95% CI)	3.1 (1.6-4.6); n=16	3 (0.8-5.2); n=7	3.2 (1.1-5.3); n=9	0.83
Bacterial/Fungal Co-infection with CV NL 63 (95% CI)	3.3 (1.7-4.9); n=17	4.7 (1.9-7.5); n=11	2.1 (0.4-3.9); n=6	0.12
Bacterial/Fungal Co-infection with CV HUK1 (95% CI)	3.5 (1.9-5.1); n=18	7.3 (3.8-10.7); n=17	0.4 (-0.3-1.1); n=1	**<0.001**
Bacterial/Fungal Co-infection with human Polyomavirus (95% CI)	4.5 (2.7-6.3); n=23	7.7 (4.1-11.3); n=18	1.8 (0.2-3.4); n=5	**0.002**
Bacterial Co-infection with WUPyV[3] (95% CI)	1.8 (0.6-2.9); n=9	3 (0.8-5.2); n=7	0.7 (-0.3-1.7); n=2	0.05
Bacterial/Fungal Co-infection with KIPyV (95% CI)	2.9 (1.4-4.4); n=15	5.1 (2.2-8); n=12	1.1 (-0.1-2.3); n=3	**0.008**
Bacterial/Fungal Co-infection with Parainfluenza virus (95% CI)	2.3 (1-3.7); n=12	3.4 (1.1-5.8); n=8	1.4 (0-2.8); n=4	0.15
Bacterial/Fungal Co-infection with PIV[4]1 (95% CI)	1.4 (0.4-2.4); n=7	1.7 (0-3.4); n=4	1.1 (-0.1-2.3); n=3	0.54
Bacterial/Fungal Co-infection with PIV2 (95% CI)	1.2 (0.2-2.1); n=6	2.6 (0.5-4.6); n=6	0 (0-0); n=0	**0.013**

Bacterial/Fungal Co infection with PIV3 (95% CI)	1 (0.1-1.8); n=5	1.7 (0-3.4); n=4	0.4 (-0.3-1.1); n=1	0.13
Bacterial/Fungal Co-infection with RSV (95% CI)	2.3 (1-3.7); n=12	2.6 (0.5-4.6); n=6	2.1 (0.4-3.9); n=6	0.76
Bacterial/Fungal Co-infection with Influenza A (95% CI)	2.1 (0.9-3.4); n=11	3.9 (1.3-6.4); n=9	0.7 (-0.3-1.7); n=2	**0.017**
Bacterial/Fungal Co-infection with Influenza B (95% CI)	1.2 (0.2-2.1); n=6	1.7 (0-3.4); n=4	0.7 (-0.3-1.7); n=2	0.30
Bacterial/Fungal Co-infection with human Bocavirus (95% CI)	1.6 (0.5-2.6); n=8	2.6 (0.5-4.6); n=6	0.7 (-0.3-1.7); n=2	0.09
Bacterial/Fungal Co-infection with HMPV(95% CI)	1.4 (0.4-2.4); n=7	2.6 (0.5-4.6); n=6	0.4 (-0.3-1.1); n=1	**0.04**
Bacterial/Fungal Co-infection with Adenovirus (95% CI)	1 (0-2); n=6	2 (0-3); n=4	1 (0-2); n=2	0.30

N=number of patients, CI[1] = Confidence interval, CV[2] = Coronavirus, PyV[3] = Polyomavirus, PIV[4] = Parainfluenza virus*p = Haematological malignancy vs Solid Tumour. Incidence** per 100 child years

6.2.4. Clinical and Laboratory Parameters for Mixed Bacterial and or Fungal Coinfections with Respiratory Viruses in Children treated for Cancer

There were no significant differences in the maximum temperature, duration of fever, median CRP and PCT, median white cell count, and absolute neutrophil, lymphocyte and monocyte counts between the overall group and cohorts with HM and ST for sole respiratory viral infection and coinfections with bacteria/fungi and respiratory viruses; data not shown.

6.2.5. Pneumonia in Children with Acute Respiratory Viral infections Treated for Cancer

Of the 528 SSE episodes, 116 (21.97%) were diagnosed as having pneumonia (82 and 34 with HM and ST respectively). Respiratory viruses were more commonly identified in sepsis cases associated with pneumonia (66.3%; 61/116) compared to those without pneumonia (49.2%; 150/412; p=0.012). The respiratory viruses most frequently associated with pneumonia were HRV (24.1%), CV HUK1 (6.5%), KIPyV (6.3%), and Adenovirus and RSV (5.3% each). Influenza A which was identified in 9.8% pneumonia SEs cases 2.6% of non-pneumonia SEs (p=0.005); Figure 3.

6.2.6. Time to Recovery of Infection by Type of Pathogen

There were no differences in time to recovery of the septic episode for infections by sole bacterial/fungal infection(p=0.14), sole respiratory viruses (p=0.163), and, respiratory viral and bacterial/fungal co-infections (p=0.376) for the cohort with HM and ST, (data not shown).

6.2.7. Factors Associated with Respiratory Viral and Bacterial Coinfections

Among children with a respiratory virus associated SSE, those who were HIV-infected (61.5%) to have concurrent bacteraemia/fungaemia compared to HIV-uninfected children

(87.9%; p=0.015); Table 21). Tumour type, nutritional status, age, gender, and treatment intensity had no association with greater percentage of children with a respiratory virus associated SSE having concurrent bacteraemia/fungaemia; Table 21.

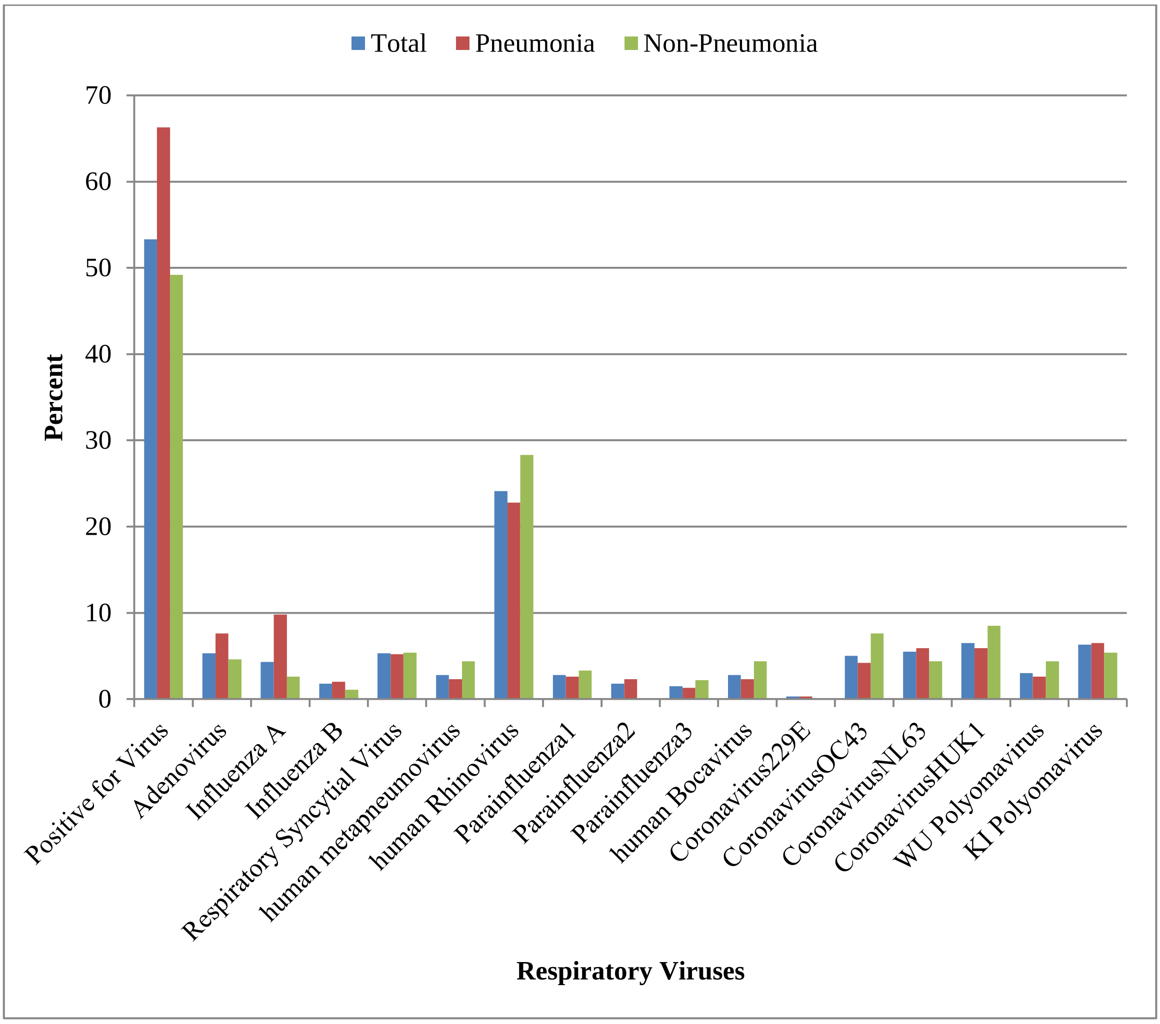

Figure 3: Respiratory Virus in Pneumonia and non-Pneumonia Septic Episodes in Children with Cancer

Table 21: Factors Associated with Sole Respiratory Viral and Respiratory Viral Bacterial/Fungal Co-infections

Parameter	Percentage of Respiratory Viral and Bacterial/Fungal Co-infections compared with Sole Respiratory Viral Infections (95% CI)	Adjusted Odds Ratio (95% CI; p-value)
Age (years)		
1-5	91.3 (78.3-97.2)	3.8 (0.8-17.6); p= 0.09
>5-10	76.9 (55.9-90.3)	1.0 (0.3-4.2); p= 0.95
>10	78.3 (55.8-91.7)	referent group
Female	92.1 (77.5-97.9)	referent group
Male	79.3 (66.3-88.4)	0.3 (0.07-1.2); p= 0.08
MUAC[1]>5th percentile	94.4 (70.6-99.7)	referent group
MUAC<5th percentile	82.1 (71.4-89.5)	0.3 (0.03-2.2); p= 0.22
AMA[2]>5th percentile	92.1 (77.5-97.9)	referent group
AMA<5th percentile	79.3 (66.3-88.4)	0.3 (0.1-1.2); p= 0.09
TSFT[3]>5th	85.0 (72.9-92.5)	referent group
TSFT<5th percentile	83.3 (66.5-93.0)	0.9 (0.3-2.8); p= 0.84
HIV-uninfected	87.9 (78.5-93.8)	referent group
HIV-infected	61.5 (32.3-84.9)	**0.2 (0.04-0.7); p= 0.015**
Solid Tumour	82.9 (65.7-92.8)	referent group
ST[4] local	80.0 (58.7-92.4)	referent group
ST metastatic	90.0 (54.1-99.5)	2.3 (0.2-22.1); p= 0.49
Haematological malignancy	85.3 (73.3-92.62)	1.2 (0.4-3.7); p= 0.76
HM[5] Medium risk	100 (65.6-100)	referent group
HM High-risk	82.4 (68.6-91.1)	0 (0-Inf); p= 0.99
Treatment intensity[6] 1	80.0 (58.7-92.4)	referent group
Treatment intensity 2	90.9 (69.4-98.4)	2.2 (0.4-14.2); p= 0.39
Treatment intensity 3	83.7 (69.8-92.2)	0.9 (0.1-8.2); p= 0.96

MUAC[1] = mid-upper arm circumference, AMA[2] = Arm muscle area, TSFT[3] = Triceps skinfold thickness, ST[4] = Solid tumour, HM[5] = Haematological malignancy, Treatment intensity[6] = Standard intensity =1, Medium intensity = 2, High intensity =3, MCSE[7] = microbiologically confirmed septic episode, SSE[7] = Suspected septic episode

6.2.8. DEATHS IN CHILDREN WITH ACUTE RESPIRATORY VIRAL INFECTIONS AND CANCER

Twelve (7.1%) children died because of sepsis with respiratory viral and concurrent bacteraemia /fungaemia. All twelve of these individuals had a HM, including (22.2%; n=4) who were HIV-infected. Eighteen viruses were identified in the 12 children who died. Additionally, at the time of death, there were multiple bacteria and fungi were isolated, including multi-drug resistant bacteria. Four who died, also had microbiologically confirmed TB. Human Coronaviruses (44.4% of 18) was the most common virus in the children who died (one CV HUK1 1, three CV OC43, and four CV NL63), followed by HRV (27.8%; n=5). Additional viruses identified among the fatal cases were HMPV (5.6%; n=1), Influenza A (11.1%; n=2), HPyV (11.1%; n=2); Table 22.

Table 22: Summary of Deaths from Respiratory Viral and Bacterial and/or Fungal Coinfections Causing Sepsis in Children Treated for Cancer

Pt	Sex	Age	Disease	HIV	Blood culture	Urine culture	Other site	Virus	Clinical Syndrome
1	M	158	NHL[1]	Pos	MRSA[5], CONS[6]	No growth	CSF[9]: MRSA Skin: MRSA	HMPV[11]	Mucositis
2	M	139	HL[2]	Neg	*Bacillus, Enterococcus faecalis*, VRE[7] *Aspergillus niger*	*Enterococcus faecalis*, ESBL *Escherichia coli*	Nil	HRV[12]	Pneumonia, Gastroenteritis, Herpes stomatitis, Mucositis
3	M	137	ALL[3]	Neg	CONS	ESBL *Escherichia coli* *Candida albicans*	Nil	HRV	Urinary tract infection
4	M	125	ALL	Neg	*Bacillus, Klebsiella pneumonia*	Not done	Sputum MTB[10]	CV[13]OC43, CVHUK1	Pneumonia, Herpes stomatitis, Proven TB
5	F	112	ALL	Neg	*Acinetobacter baumanni*, *Empedobacter brevis*	ESBL *Pseudomonas aeruginosa*	Nil	CVNL63	Pneumonia, Gastroenteritis, Urinary tract infection
6	F	100	NHL	Pos	No growth	Not done	Sputum: MTB	Flu[14]A, HRV, CVOC43, CVNL63	Pneumonia, Herpes stomatitis, Mucositis, Proven TB
7	F	99	ALL	Neg	*Candida albicans*	No growth	Nil	WUPyV[15]	Mucositis
8	F	98	ALL	Neg	ESBL[8] *Klebsiella pneumonia*	No growth	Sputum: MTB	CVNL63,	Pneumonia, Gastroenteritis, Proven TB

								KIPyV	
9	M	50	NHL	Pos	CONS	*Escherichia coli*	Nil	HRV	Pneumonia, Gastroenteritis, Herpes stomatitis, Mucositis, Urinary tract infection, Probable TB
10	F	44	ALL	Neg	No growth	ESBL *Klebsiella pneumoniae*	*Candida albicans*	CVOC43	Pneumonia, Herpes stomatitis, Fungal urinary tract infection, Probable fungal sepsis
11	M	23	NHL	Pos	ESBL *Escherichia coli*	No growth	Sputum : MTB	HRV, CVNL63	Herpes stomatitis, Mucositis, Probable fungal sepsis
12	M	21	AML[4]	Neg	*Enterococcus faecalis*	*Enterococcus faecalis*	Nil	FluA	Pneumonia, Gastroenteritis, Herpes stomatitis, Mucositis, Probable TB, Probable fungal sepsis

NHL[1] = Non-Hodgkin's lymphoma, HL[2]= Hodgkin's lymphoma, ALL[3] = Acute lymphoblastic leukaemia, AML[4] =Acute myeloid leukaemia, MRSA[5] =Methicillin-resistant *Staphylococci*, CONS[6] =Coagulase-negative *Staphylococci*, VRE[7] =Vancomycin-resistant *Enterococci*, ESBL[8] =extended spectrum β-lactamase producer, CSF[9] =cerebrospinal fluid, MTB10 =*Mycobacterium tuberculosis*, HMPV[11] =human Metapneumovirus, HRV[12] =human Rhinovirus, CV[13] =human Coronavirus, FluA[14] =Influenza A virus, PyV[15]=human Polyoma virus

6.3. DISCUSSION

Our study documented that a high percentage (53.1%) of suspected septic episodes were associated with identification of a respiratory virus, both in children with HM (57.1%) and ST (47.2%). These findings are similar to other studies in children with cancer, but the panel of respiratory viruses did not include all the viruses we investigated for, where respiratory viruses have been identified from 44% to 57% of children with febrile neutropenia (Benites et al., 2014, Koskenvuo et al., 2008, Torres et al., 2012b). Sixty-four (47.2%) of SSEs in children with ST were associated with at least one respiratory virus. We identified very few previously published studies that investigated the role of respiratory viral infections in children with ST, independently of inclusion with HM cases. A recent study reported that patients with ST had significantly more respiratory viral infections (53%) but seemed to tolerate them better and required shorter hospitalisation than children with HM (Christensen et al., 2005). A study of influenza in a paediatric oncology subpopulation, reported that 5.3% influenza-documented illnesses occurred in children with cancer and more than a third of these had been diagnosed with ST (Tasian et al., 2008).

In our study the most frequently identified respiratory viruses were HRV (26%), HCoV (18%) and, HPyV (11%). Other studies also documented a high frequency of HRV ranging from 31% (Benites et al., 2014) to 59% (Suryadevara et al., 2012b). In a cohort of South African children without cancer, HRV was the most prevalent in HIV-infected (31.7%) and HIV-uninfected children (32.0%), followed by CV OC43 (12.2%) and HBoV (9.5%) in HIV-I; and by HBoV (13.3%) and WUPyV (11.9%) in HIV-uninfected children (Nunes et al., 2014). A prospective case-control study from the Netherlands, to investigate the prevalence of respiratory viruses in asymptomatic and symptomatic young non-cancer children study showed that the prevalence of respiratory viruses is high in asymptomatic children (27%), particularly in infants (44%). Studies using a large panel of respiratory viruses (Kusel et al., 2006) have reported a prevalence of 25% in prospectively followed infants during periods without symptoms, and Jartti et al.(Jartti et al., 2008) found at least one virus in 45% of asymptomatic infants younger than 1 year old.

There is conflicting data about the significance of HRV as an aetiological agent for sepsis, in children with or without cancer. An Italian study was undertaken in young children to evaluate HRV infection and factors involved in disease severity. The study documented that HRV was a common pathogen (71%) in mild disease and was involved in 29% of cases with severe LRTI. Co-infection with RSV was the main contributor to disease severity (Costa et al., 2014). A study to investigate the impact of human Rhinovirus on morbidity and mortality outcomes in children with severe viral respiratory infection documented 32% of children with HRV infection required mechanical ventilation with a mortality of 2.1%. An immune-compromised state and bacterial co-infections were associated with mortality in this cohort. The study concluded substantial morbidity associated with severe respiratory infection due to HRV in children (Spaeder et al., 2015).

Previous studies in children with cancer have shown that HCoVs are relatively rare, accounting for less than five to eight percent of all detected respiratory viruses (Torres et al., 2012a, Benites et al., 2014). Our study, however, documented HCoVs to be the second most frequent respiratory virus. In South African children without cancer, HCoV was the second most commonly detected respiratory virus in HIV-infected children (12.2%) (Nunes et al., 2014). In a report from Brazil in children with cancer, HCoV was the most commonly identified respiratory virus, although HCoV was most commonly identified in children with multiple virus infections (Alvares et al., 2014). A study from the USA to documented a 7.6% prevalence of HCoV species among non-cancer children hospitalized for acute respiratory illness and/or fever and a prevalence of 7.1% in asymptomatic controls. The study concluded that in children hospitalized for acute respiratory illness and/or fever, HCoV infection was not associated with hospitalization or with increased severity of illness (Prill et al., 2012).

In our study, PIV accounted for six percent of the respiratory viruses. This finding is in contrast to other studies in which PIV3 was the most prevalent virus in children with cancer. Maeng et al documented 6.4% of children with cancer had a respiratory viruses isolated during a febrile illness; of these 54% were PIV, with PIV3 the predominant virus. The overall mortality from PIV infection was 23% (Maeng et al., 2012, Srinivasan et al., 2011). However, this study did not investigate for all the viruses in our study panel.

Globally, RSV is the most common cause of childhood acute LRTI and a major cause of admission to hospital because of severe acute LRTI in predominantly otherwise healthy children. Mortality data suggest that RSV is an important cause of death in childhood from acute LRTI (Nair et al., 2010). In our study cohort, 5.6% of all SSE episodes were associated with RSV infection, 8.4% of bacteraemic /fungaemic episodes were co-infected with RSV, and RSV was identified together with HRV in six SEs. None of the patients in our cohort who had RSV identified, died. This finding is similar to that reported from Brazil in which the prevalence of RSV was 8.7%, although none of the children with RSV identified, died (Benites et al., 2014). In contrast, another study followed 59 high-risk paediatric cancer patients identified with RSV infection. One third had pneumonia with a mortality rate of 5%. Lymphopenic and male children progressed from a URTI to a LRTI (Chemaly et al., 2014). In contrast to HRV, RSV is seldom encountered in control patients, which suggests that a positive RSV test result is usually of clinical significance (Jansen et al., 2011) .

None of the patients in our cohort received Influenza A immunization. It has been documented that cancer increases the risk of influenza complications and mortality can reach up to 9% in cancer patients undergoing active therapy (Loulergue et al., 2008). Cancer patients are a high-risk group who are prone to post-influenza complications, therefore should be immunized against influenza before every seasonal epidemic (Shehata and Karim, 2014).

However, it is currently unclear whether patients undergoing systemic chemotherapy can achieve adequate serologic responses to vaccines. Patients undergoing treatment for cancer are at increased risk of infection, for which the influenza vaccine may offer additional protection and significant benefit. A Cochrane review in paediatric oncology patients receiving chemotherapy concluded that children are able to generate an immune response to the influenza vaccine, but it remains unclear whether this immune response protects them from influenza infection or its complications (Goossen et al., 2013). The Infectious Disease Society of North America recommends that children with cancer should be treated with the antiviral agent, Tamiflu (Harper et al., 2009), though none of the children in our cohort were treated prophylactically or therapeutically with Tamiflu. Additionally, protective measures e.g. adequate infection control, hand washing, immunization of close contacts, medical, nursing and hospital staff, early investigation, and treatment with Tamiflu for suspected cases of Influenza must be considered.

Overall, 17.5% of SEs in our study cohort had viral co-infections, which is similar to that reported by others (17%-19.7%) (Koskenvuo et al., 2008, Benites et al., 2014), with HRV (23.1%) and RSV (8.7%%) having the highest rates of co-infection. Similar rates for viral-viral co-infections are also observed in children without immunosuppression (14% to 44%), with HRV being the second or third most frequent virus (Sly and Jones, 2011). Co-detection of multiple viruses alone was, however, not associated with severity of the illness or death. This, as well as identification of some of respiratory viruses in general, could be due to identification of these viruses either being co-incidental to the clinical illness, or self-limiting in their clinical course even among immunocompromised children with underlying cancer. It may also reflect prolonged excretion of these viruses (Jansen et al., 2011). The relationship between immunosuppression in children with cancer and viral shedding is not clear. Detection of CV HUK1 and CV 229E in respiratory specimens of transplanted children were reported for at least 38 days and 11 weeks, respectively, possibly suggesting that immunocompromised children may have a prolonged duration of shedding of these respiratory viruses (Song et al., 2010). Such prolonged shedding in immunocompromised individuals may result in a greater frequency of identification of these respiratory viruses when investigated for respiratory illness. This may influence greater nosocomial transmission of these respiratory viruses because of children with cancer and febrile illnesses are hospitalized for treatment and monitoring. Nosocomial respiratory viruses add to the burden in children's hospitals and increase the risk of transmission, especially to immune-compromised children. Early identification of respiratory viruses in asymptomatic children will enable effective isolation in hospital with adequate isolation facilities, especially for immunocompromised children.

There are conflicting reports on the significance of viral-viral co-infections in children. A study from Italy, in children without immunosuppression documented those with viral-viral co-infections more frequently required hospital admission than those with single respiratory viruses (Cilla et al., 2008), although specific viral combinations may impact on the clinical relevance of the viral-viral co-infections.

HRV was the most frequent respiratory virus in combination with other respiratory viruses, (23 SEs with HCoV, 10 with HPyV, 8 each PIV with ADV, 7 with CV NL63, 6 each with RSV and KIPyV). Human Rhinovirus (17.3%) was the most frequent virus in co-infections, followed by HCoV (11%), and HPyV (5.3%). In our study, there were no statistically significant differences for HRV as a sole respiratory virus or as part of a co-infection. However, CV 229E and CV OC43 (20 SEs with co-infection) did not occur as sole respiratory virus and only occurred in SEs with co-infections. CV NL63 was detected twice as a sole respiratory virus and in 20 SEs with co-infection, and, CV HUK1 was identified four times as a sole respiratory virus and in 22 SEs as part of a co-infection. HPyV was isolated in 35 SEs, 29.41% as a sole respiratory virus and in 14.04% of co-infections. Additionally, KIPyV was isolated in 25 SEs as a sole viral pathogen. Certain respiratory viruses such as PIV, HBoV, HMPV, and RSV, have been reported to be more significantly associated with the presence of co-infections (Diaz et al., 2015). RSV, HMPV, and HBoV have been reported to have a higher frequency of infections with more than one virus (da Silva et al., 2013). In a study from Chile in paediatric oncology patients, RSV (31.17%), HRV (24.68%) and HBoV (12.99%) were most frequently iden-tified in co-infections, while RSV(35.58%), HRV (24.04%) and Influenza A(14.42%) were the most common respiratory viruses detected as sole respiratory virus (Torres et al., 2012b).

Thirty five percent of the respiratory virus associated SSEs had concurrent bacteraemia-/fungaemia in our study, which was more common in children with HM (33.9%) than in children with ST (22.7%). Children with concurrent respiratory virus infection and bacteraemia/fungaemia in our study had a greater depth of pancytopenia with SEs than those with culture negative and respiratory virus negative sepsis. Severe lymphopenia has been documented as a risk from for progression to LRTIs and death in children with RSV infection (El Saleeby et al., 2008b). In adult patients who were transplanted for leukaemia, the risk factors for respiratory viral infections were neutropenia and lymphopenia, and fatality was documented in those with absolute lymphocyte count of < 200 cells/µL in those patients who had influenza, but the study did not document if these individuals had concurrent bacteraemia /fungaemia (Chemaly et al., 2006). A study from Brazil in children with cancer (Benites et al., 2014) documented 23.5% with leukopenia, 20.5% with neutropenia, 16.7% with severe neutropenia and 41% of patients with lymphopenia had respiratory viruses identified during a febrile illness. None of the febrile episodes in this study had respiratory viral and bacterial/fungal co-infections. Not many studies have documented respiratory viral and bacterial/fungal co-infections in children undergoing cancer therapy. A study from Chile reported 33% of respiratory viral infections had co-infections with bacteraemia (Torres et al., 2012b).

We documented bacterial/fungal and respiratory viral co-infections were associated with a greater depth of neutropenia, lymphopenia, and monocytopenia than those with culture negative and respiratory viral negative sepsis. The Chilean study found no differences between neutropenia, lymph-openia and monocytopenia between sole viral or co-infections with bacteria/fungi and respiratory viruses (Torres et al., 2012b). However, this study did not explore the relationship between depth and duration of pancytopenia and association to co-infections with respiratory viruses and bacteraemia /fungaemia.

In our study, biomarkers were not useful to discriminate between SSEs with respiratory viral and bacterial co-infections and those without. Biomarkers provide information about the host response to pathogens (bacteria, virus or fungi) causing infection. There is rising evidence that different microorganisms may promote different inflammatory responses, and levels of some biomarkers such as CRP and PCT are associated with distinct aetiological pattern. CRP and PCT show higher levels in bacterial pneumonia than pneumonia caused by respiratory viruses (Cillóniz et al., 2015). However, this was not the case in our study.

In our study, respiratory viruses were frequently associated with pneumonia. The respiratory viruses most frequently associated with pneumonia were human Rhinovirus (28.26%), human Coronavirus (20.88%), human Polyomavirus (9.9%), and Influenza A virus (9.78%). In our study, none of the respiratory viruses showed a significant relationship as aetiological agents for pneumonia as they were also detected in non-pneumonia cases, except for Influenza A which was identified as a possible aetiological agent for pneumonia in 10% of the episodes of pneumonia ($p=0.005$).

Twelve patients of the overall thirteen in the cohort who died because of sepsis had bacteraemia/ fungaemia (seven multi-drug resistant bacterial pathogens) and respiratory viral co-infections. Eighteen different viruses were identified in the twelve patients, including two with Influenza A and four patients had microbiologically confirmed TB. The presence of a respiratory virus and its clinical significance and relationship with outcome is uncertain as none of the patients who died had post-mortems, which would have helped to delineate the microbiological cause of death. Some studies reported less severe infections and no fatal outcomes in children with cancer diagnosed with respiratory viruses (Suryadevara et al., 2012b, Benites et al., 2014). Others have reported severe infections with fatal outcomes (Christensen et al., 2005, Srinivasan et al., 2013). Among the respiratory viruses, RSV is of particular concern for resultant mortality in high-risk patients. In immunocompetent patients, RSV is often a self-limiting URTI; however, in patients with AML and haemopoietic stem cell recipients, RSV can progress to a LRTI. In this setting RSV is associated with a 14% case fatality rate in patients with AML and a 50% case fatality rate in paediatric recipients of haemopoietic stem cell transplantation (Sung et al., 2007, El Saleeby et al., 2008b).

Multiple potential pathogens, including viruses, bacteria, fungi, and MTB were identified from the children who died of sepsis in our study. The deaths in patients with multiple co-infections suggest a more severe clinical context and a may be explained by the prolonged and profound pancytopenia documented in this cohort of patients. In future, post-mortem studies may benefit in identifying the exact microbiological cause of death.

The result of our study indicate that further studies are essential with asymptomatic children with cancer controls, to help us understand the significance of respiratory viral infections either as sole agents or as part of viral-viral co-infections or viral bacterial/fungal co-infections and its role in sepsis among children with cancer. We also need to study interactions between specific respiratory viruses, and to identify whether the severe acute infection is related to the underlying immunological status, type of cancer or the respiratory virus. Accurate and timely diagnosis of respiratory viral infections in children has potential benefits by reducing excessive antibiotic use.

All children in the study cohort received antimicrobials; therefore, we could not investigate the clinical course of respiratory viral infection in the absence of antibiotic use. Although detection of a respiratory virus in a child with a febrile illness cannot influence empirical antimicrobial therapy, studies are necessary to understand the relationship between respiratory viral infections and the aetiology of the febrile illness episode.

Limitations of our study, and others, with regard to the identification of respiratory viruses include that detection of respiratory virus in nasopharynx by molecular techniques does not necessarily indicate respiratory disease. Future studies may consider determining viral load in sequential samples and correlating findings with clinical signs and symptoms.

7. Infectious Complication in HIV-Infected and HIV-uninfected Children Treated for B-Cell Non-Hodgkin's Lymphoma

7.2. Introduction

Since the implementation of the antiretroviral (ARV) roll out programme in South Africa, HIV-infected children with NHL, are treated with ARVs and, treated similarly to HIV-uninfected children. There are, however, a number of challenges to chemotherapy treatment in HIV-infected children, including that cancer therapy compounds an already immune-suppressed state in these children. There are no established treatment guidelines in both adult and paediatric populations for the treatment of HIV-related NHL. A number of issues remain to be addressed; including whether treatment should be palliative or curative, the effect of ARVs and cancer drug interactions and the effect of the underlying HIV on the susceptibility to severe and fatal infections. There are currently no published studies, to our knowledge, that have compared the treatment-associated morbidity in HIV-infected compared to HIV-uninfected children with NHL. Because of the underlying organ dysfunction including that of the bone marrow, impaired immune system and the need for multiple drugs to treat HIV infection, it is assumed that HIV-infected children are less likely to tolerate standard chemotherapy (Mueller, 1999).

7.3. Aim of the Study

This retrospective study aimed to establish the infectious complications in HIV-infected and HIV-uninfected children treated for NHL with the same chemotherapy treatment regimen: Appendix 11. We also compared the stage of disease (Ann Arbor system), the degree and depth of bone marrow suppression and its association with infectious complications.

7.4. METHODS

Hospital records for HIV-infected and HIV-uninfected children with NHL were retrospectively reviewed for the period 2000 to 2009. The following variables: age at diagnosis, HIV status, stage of cancer, sex, and, anthropometric measures at presentation. In addition, the CD4+ lymphocyte number and percentage, and HIV-1 viral load was documented for HIV-infected children. All HIV-infected children were initiated on ARVs; Appendix 13. The statistical methods employed are described in Chapter 3.

Data on septic events noted in the patient records were abstracted onto a standardised form, including maximum temperature, duration of fever, white cell count, absolute neutrophil, lymphocyte and monocyte counts, CRP, blood culture results, urine culture results and associated clinical syndromes such as pneumonia and tuberculosis at the time of the sepsis episode.

7.5. RESULTS

7.5.1. DEMOGRAPHICS OF HIV-INFECTED AND HIV-UNINFECTED CHILDREN WITH NON-HODGKIN'S LYMPHOMA

Fifty-eight children with NHL were identified, 30 (51.7%) of whom were HIV-infected. The majority of children (80.7%) were males and the median age at diagnosis was 89.5 months; which was not significantly different between both groups. HIV-infected children were generally more malnourished than HIV-uninfected children were, albeit only significant for the HFA Z-score (p=0.046); Table 23.

7.5.2. ADMISSION PARAMETERS ASSOCIATED WITH MICROBIOLOGICALLY CONFIRMED SEPSIS

Children one to five years old had an incidence of 84 per 100 child years of micro-biologically confirmed sepsis (MSCE) which was 2.4 fold greater comparted to in children >10 years of age (p<0.001; Table 24). The incidence of MCSE was 2.2 fold greater in HIV-infected (95 per 100 child years) than in HIV-uninfected children ($p<0.001$). Male children had a lower incidence (60 per 100 child years) of MCSE than females ($p=0.021$).

7.5.3. Comparison of Septic Events in HIV-Infected and HIV-Uninfected Children with Non-Hodgkin's Lymphoma

The person-time of follow-up was 128.8 and 56.3 per 100 child years among the HIV-uninfected and HIV-infected children, respectively; Table 25. During this time, there was a higher incidence (per 100 child years) of suspected sepsis episodes (SSE) among the HIV-infected than HIV-uninfected cases (170 vs. 80, $p<0.001$). Overall, 84% of SSE were associated with a positive blood culture, which did not differ by HIV-status; Table 25. Among HIV-infected children, no significant associations were identified between HIV-1 viral load and frequency of fungaemia, and Gram-negative bacterial pathogens causing urinary tract infections.

There was a higher incidence (per 100 child years) of MCSE among HIV-infected (122.5) than HIV-uninfected children (48.2), including higher incidence of Gram-positive bacteraemia (121 vs. 35, $p<0.001$) and Gram-negative bacteraemia (101 vs. 31, $p<0.001$); Table 25.

The incidence (per 100 child years) of polymicrobial infections with Gram-positive and Gram-negative bacteraemia (43 vs 19; $p<0.001$), and Gram-positive bacteraemia plus Gram-negative bacteraemia plus fungaemia (7 vs. 1; $p=0.018$) were also more frequent in HIV-infected than HIV-uninfected children. There were more pathogens per septic event isolated by blood culture in HIV-infected (1.4) than in HIV-uninfected children (0.9; $p= 0.005$). Similarly there were more GPB (0.71 vs 0.43; $p=0.025$) and GNB (0.59 vs 0.39; $p=0.025$) per septic event in HIV-infected than in HIV-uninfected children with sepsis.

Table 23: Demographics of HIV-Infected and HIV-Uninfected Children with Non-Hodgkin's Lymphoma

Characteristic	Overall N=58	HIV-Infected N=30	HIV-Uninfected N=28	P*value
Male	46 (80.7%)	24(80.0%)	22(81.5%)	0.88
Mean Age (months) (SD[1])	89.5 (53.3)	96.3 (51.2)	82.1 (55.5)	0.32
Median LDH[2] (μ/L) (SD)	1044(708-2269)	875(678-1973)	1124(780-2760)	0.23
Stage 2	2 (3.6%)	0(0%)	2(7.7%)	-
Stage 3	41 (73.2%)	20(66.7%)	21(80.8%)	-
Stage 4	13 (23.2%)	10(33.3%)	3(11.5%)	0.05
WFH[3] Z-score (SD)	-0.78(1.46)	-1.12(1.46)	-0.58(1.48)	0.39
WFA[4] Z-score (SD)	-1.46 (1.32)	-1.74(1.44)	-1.21(1.18)	0.21
HFA[5] Z-score (SD)	-1.89(1.40)	-2.24(1.29)	-1.48(1.45)	0.05
BMI[6] Z-score (SD)	-0.68(1.57)	-0.98(1.40)	-0.14(1.74)	0.09

N= number of patients, SD[1] = Standard deviation, LDH[2] = Lactate dehydrogenase enzyme, WFH[3] = Weight-for-Height, WFA[4] = Weight-for-Age, HFA[5] = Height-for-Age, BMI[6] = Body mass index, *p= HIV-infected vs HIV-uninfected

Table 24: Admission Parameters Association with Sepsis Children with Non-Hodgkin's Lymphoma

Parameter	Incidence Rate (95% CI)	Adjusted Incidence Rate Ratio (95% CI; p-value)
Age (years)		
1-5	84 (63-105)	**2.4 (1.56-3.7); p<0.001**
>5-10	58 39-76)	1.1 (0.7-1.84); p= 0.61
>10	41 26-55)	referent group
Female	96 (57-135)	referent group
Male	60 (48-71)	**0.6 (0.4-0.9); p= 0.021**
HIV-uninfected	44 (33-55)	referent group
HIV-infected	95 (72-117)	**2.2 (1.5-3.1); p<0.001**

7.5.4. Clinical Syndromes Associated with Sepsis In HIV-Infected and HIV-Uninfected Children treated for NHL

The overall incidence (per 100 child years) of pneumonia was 31, and was higher in HIV-infected (75) than HIV-uninfected children were (12; p<0.001). Similarly, there was a higher incidence of tuberculosis (25 vs 2; p<0.001), invasive fungal infections (28 vs 7; p=0.001), Herpes stomatitis (137 vs 44; p<0.001), and gastroenteritis (39 vs 15; p=0.004) in HIV-infected than HIV-uninfected children; Table 26. Clinical syndromes in HIV-infected children were associated with higher incidence ratio rates (IRR). The IRR in the HIV-infected cohort for TB was 10.8, for pneumonia 6.5, for invasive fungal infections 9.2, for Herpes stomatitis 3.1, and for gastroenteritis, 2.7.

7.5.5. CLINICAL AND LABORATORY PARAMETERS OF SUSPECTED AND MICROBIOLOGICALLY CONFIRMED SEPSIS IN HIV-INFECTED AND HIV-UNINFECTED CHILDREN WITH NON-HODGKIN'S LYMPHOMA

Generally, the clinical presentation of SSE and MCSE were similar between HIV-infected and HIV-uninfected children, including CRP and PCT levels. The mean maximum temperature was, however, higher in the HIV-infected with MCSE ($p=0.022$; Table 26). In addition, HIV-infected children with SSE had a longer duration of neutropenia ($p<0.001$) and similarly, the duration of lymphopenia was longer in the HIV-infected group with SSE ($p<0.001$) and for MCSE ($p=0.001$) than in HIV-uninfected children, Table 27.

Table 25: Comparison of Septic Events in HIV-Infected and HIV-Uninfected Children Treated for Non-Hodgkin's Lymphoma

Parameter	Overall; N=58	HIV-Infected; N=30	HIV-Uninfected; N=28	p-value[4]
Person time observation (years)	185	56.	128.	NA
Number children with SSE[1] (%)	57 (98.3%)	30(100.0%)	27(96.4%)	0.79
Number of SSE	199	96	103	NA
Mean number of SSE per patient (SD)	3.43 (2.04)	3.20 (1.85)	3.68 (2.23)	0.38
SSE episodes per 100 child year (95% CI)	107.5 (92.6-122.5); n=199	170.4 (136.3-204.5); n=96	80 (64.6-95.4); n=103	**<0.001**
At least one positive blood culture per SSE (%)	49 (84.5%)	26 (86.7%)	23 (82.1%)	0.78
Mean number of pathogens in blood per SSE (SD)	1.14 (1.23)	1.40 (1.40)	0.90 (1.00)	**0.005**
Mean number of Gram-positive bacteria in blood per SSE(SD)	0.56 (0.86)	0.71 (0.97)	0.43 (0.72)	**0.025**
Mean number of Gram-negative bacteria per SSE (SD)	0.48 (0.66)	0.59 (0.69)	0.38 (0.61)	**0.025**
Mean number of Fungi per SSE (SD)	0.09 (0.29)	0.09 (0.29)	0.09 (0.28)	0.86
Total death due to sepsis (%)	12 (20.69%)	10(33.33%)	2 (7.14%)	**0.022**
MCSE[2] (95% CI)	70.8 (58.7-82.9); n=131	122.5 (93.6-151.4); n=69	48.2 (36.2-60.1); n=62	**<0.001**

Incidence Gram-positive bacteraemia (95% CI)[3]	61.1 (49.8-72.3); n=113	120.7 (92-149.4); n=68	35 (24.7-45.2); n=45	**<0.001**
Incidence Gram-negative bacteraemia (95% CI)	52.4 (42-62.8); n=97	101.2 (74.9-127.5); n=57	31.1 (21.4-40.7); n=40	**<0.001**
Incidence fungaemia per 100 child year (95% CI)	9.7 (5.2-14.2); n=18	16 (5.5-26.4); n=9	7 (2.4-11.6); n=9	0.09
Incidence[3] Mixed Gram-positive + Gram-negative bacteraemia (95% CI)	18.9 (12.6-25.2); n=35	42.6 (25.6-59.6); n=24	8.5 (3.5-13.6); n=11	**<0.001**
Incidence Mixed Gram-positive bacteraemia + Fungaemia (95% CI)	4.3 (1.3-7.3); n=8	10.7 (2.1-19.2); n=6	1.6 (0-3.7); n=2	**0.008**
Incidence Mixed Gram-negative Bacteraemia + Fungaemia (95% CI)	4.9 (1.7-8); n=9	8.9 (1.1-16.7); n=5	3.1 (0.1-6.2); n=4	0.11
Incidence Mixed Gram-positive + gram-negative bacteraemia + Fungaemia (95% CI)	2.7 (0.3-5.1); n=5	7.1 (0.1-14.1); n=4	0.8 (0-2.3); n=1	**0.018**

N=number of patients, SSE[1] = Suspected Septic Episode, MCSE[2] = Microbiologically-Confirmed Septic Episode, [3] Incidence per 100 child years. [4]p = HIV-infected vs HIV-uninfected, Incidence [5] number of bacteria and fungi included in individual analysis

Table 26: Incidence (per 100 Child Years) of Clinical Syndromes Associated with Sepsis in HIV-infected and HIV-uninfected Children Treated for Non-Hodgkin's Lymphoma

Parameter	Overall N=185	HIV-Infected N=56	HIV-Uninfected N=128	IRR (95% CI)	P*-value
Incidence** of Pneumonia (95% CI)	30.8 (22.8-38.8); n=57	74.6 (52-97.1); n=42	11.7 (5.8-17.5); n=15	6.5 (3.5-12.5)	**<0.001**
Incidence of Tuberculosis (95% CI)	9.2 (4.8-13.6); n=17	24.9 (11.8-37.9); n=14	2.3 (0-5); n=3	10.8 (3-58.3)	**<0.001**
Incidence of Proven Tuberculosis (95% CI)	6.5 (2.8-10.2); n=12	17.8 (6.7-28.8); n=10	1.6 (0-3.7); n=2	11.5 (2.5-108.1)	**<0.001**
Incidence of Probable Tuberculosis (95% CI)	2.7 (0.3-5.1); n=5	7.1 (0.1-14.1); n=4	0.8 (0-2.3); n=1	9.2 (0.9-453.8)	**0.018**
Incidence of Fungal infections (95% CI)	13.5 (8.2-18.8); n=25	28.4 (14.5-42.3); n=16	7 (2.4-11.6); n=9	4.1 (1.7-10.5)	**0.001**
Incidence of Herpes stomatitis (95% CI)	72.4 (60.1-84.7); n=134	136.7 (106.2-167.2); n=77	44.3 (32.8-55.8); n=57	3.1 (2.2-4.5)	**<0.001**
Incidence of Gastroenteritis (95% CI)	22.2 (15.4-28.9); n=41	39.1 (22.7-55.4); n=22	14.8 (8.1-21.4); n=19	2.7(1.4-5.2)	**0.004**

N=total person-years, n= number of events, *p- value compare HIV-infected vs. HIV-uninfected children, Incidence** reported per 100 child years

Table 27: Clinical and Laboratory Parameters of Suspected Septic Episodes and Microbiologically Confirmed Sepsis in HIV-Infected and HIV-Uninfected Children with Non-Hodgkin's Lymphoma

	Overall			Suspected Septic Episode			Microbiologically Confirmed Septic Episode		
Parameter	HIV-Infected N=30	HIV-Uninfected N=28	P*	HIV-Infected N=30	HIV-Uninfected N=28	P**	HIV-Infected N=30	HIV-Uninfected N=28	P***
Mean maximum Temperature °C (SD[1])	38.9 (0.44)	38.8 (0.37)	**0.031**	38.9 (0.47)	38.8 (0.49)	0.69	38.9 (0.44)	38.7 (0.29)	**0.022**
Duration of Fever (days) (IQR[2])	4.0 (3.0 - 6.0)	4.0 (2.0 - 6.0)	0.81	5.0 (2.3 - 5.8)	3.0 (2.8 - 5.0)	0.36	4.0 (3.0 - 6.0)	4.0 (2.0 - 7.0)	0.29
Median CRP[3] mg/L (IQR)	67.5 (34.5 - 105.5)	75.0 (43.5 - 110.3)	0.30	79.0 (28.5 - 125)	75.5 (48.8 - 113)	>0.99	66.5 (37.0 - 99.8)	75.0 (40.5 - 109)	0.27
Median WCC[4] x 10^9 cells/µL (IQR)	0.6 (0.3 - 1.2)	0.9 (0.2 - 5.8)	0.40	0.7 (0.4 - 1.0)	0.8 (0.1 - 2.4)	0.96	0.6 (0.3 - 1.2)	0.9 (0.3 - 5.9)	0.38
Median neutrophil count cells/µL (IQR)	90.0 (90.0 - 528)	90.0 (90.0 - 2977)	0.08	90.0 (90.0 - 413)	104 (90.0 - 570)	0.42	90.0 (90.0 - 573)	90.0 (90.0 - 3237)	0.13
Median lymphocyte count cells/µL (IQR)	90.0 (90.0 - 467)	90.0 (90.0 - 1000)	0.18	90.0 (90.0 - 433)	100 (90.0 - 497.0)	0.32	90.0 (90.0 - 487)	90.0 (90.0 - 1000)	0.35
Median monocyte count cells/µL (IQR)	90.0 (90.0 - 1645)	90.0 (90.0 - 670)	0.12	90.0 (90.0 - 164)	90.0 (90.0 - 659)	0.39	90.0 (90.0 - 148)	90.0 (90.0 - 600)	0.23
Duration of neutropenia[5] during a SE[6] (days) (IQR)	9.0 (5.0 - 11.8)	8.0 (6.0 - 10.8)	0.80	9.0 (8.0 - 17.5)	8.0 (6.3 - 10.0)	**<0.001**	9.0 (4.0 - 10.0)	8.5 (6.0 - 11.3)	0.29
Duration of lymphopenia[7] during a SE (days) (IQR)	10.0 (7.0 - 15.0)	8.0 (9. 0 -31.0)	**<0.001**	20.5 (12.0 - 36.8)	10.5 (6.0 - 16.5)	**<0.001**	15.0 (8.5 - 27.0)	10.0 (7.0 - 14.5)	**0.001**

N=number of patients, SD [1]= Standard deviation, IQR[2] =inter-quartile range, CRP[3] =C-reactive protein, WCC[4] = White cell count, Neutropenia [5] = Absolute neutrophil count < 1000 cells/µL, SE[6] = Septic episode, Lymphopenia [7] = Absolute lymphocyte count < 1000 cells/µL *p = overall sepsis HIV-infected vs HIV-uninfected, **p = Suspected sepsis in HIV-infected vs HIV-uninfected, ***p = microbiologically confirmed sepsis for HIV-infected vs HIV-uninfected

7.5.6. Gram-Positive Bacteria Causing Sepsis In HIV-Infected and HIV-Uninfected Children treated for Non-Hodgkin's Lymphoma

Overall, there were 61 Gram-positive MCSE per 100 child years, 121 in HIV-infected and 35 in HIV-uninfected children (p<0.001); Table 28. The most frequently identified Gram-positive bacterial iso-lates were *Streptococcus* species (n=15; 25.9%), *Enterococcus faecium* (n=14; 24.1%), and *Staphylo coccus aureus* and *Streptococcus viridans* (n=13; 22.4%) each.

The overall incidence (per 100 child years) of *Enterococcus faecium* was eight, which was higher among HIV-infected (16) than HIV-uninfected children were (four; p=0.009). Similarly, there was a higher incidence of Vancomycin- resistant *Enterococci* (seven vs 1; p=0.018) and *Staphylococcus aureus* (14 vs 4; p=0.02) sepsis episodes among HIV-infected than HIV-uninfected children; Table 28

7.5.7. Gram-Negative Bacteria Causing Sepsis in HIV-Infected and HIV-Uninfected Children treated for Non-Hodgkin's Lymphoma

Overall, there were 52 Gram-negative MCSE per 100 child years, 101 in the HIV-infected cohort and 31 in the HIV-uninfected cases (p<0.001); Table 29. The most frequently identified Gram-negative bacterial isolates were *Klebsiella* species (n=18; 23.7%), ESBL *Klebsiella* (n=14; 18.4%), *Acinetobacter baumanni* (n=10, 13.2%) and *Escherichia coli* (n=9; 11.8%).

The overall incidence (per 100 child years) of *Acinetobacter baumanni* was five, which was higher among HIV-infected (11) than HIV-uninfected children were (three; p=0.049). Similarly, there was a higher incidence of *Pseudomonas aeruginosa* (11 vs 2; p=0.022), *Stenotrophomonas maltophilia* (nine vs 1; p=0.006), *Bacillus* species (five vs 0; p=0.018), and *Salmonella* species (five vs 0; p=0.015) sepsis among HIV-infected than HIV-uninfected children; Table 29.

Table 28: Incidence (per 100 Child Years) of Gram-Positive Bacteria Causing Sepsis in HIV-Infected and HIV-Uninfected Children Treated for Non-Hodgkin's Lymphoma

Gram-Positive Bacteria	Overall N=185	HIV-Infected N=56	HIV-Uninfected N=128	P* value
Incidence** of *Streptococcus* species (95% CI)	8.1 (4-12.2); n=15	14.2 (4.4-24); n=8	5.4 (1.4-9.5); n=7	0.07
Incidence of *Enterococcus faecium* (including VRE[1])(95% CI)	7.6 (3.6-11.5); n=14	16 (5.5-26.4); n=9	3.9 (0.5-7.3); n=5	**0.009**
Incidence of VRE(95% CI)	2.7 (0.3-5.1); n=5	7.1 (0.1-14.1); n=4	0.8 (0-2.3); n=1	**0.018**
Incidence of *Staphylococcus aureus* (including MRSA[2]) (95% CI)	7 (3.2-10.8); n=13	14.2 (4.4-24); n=8	3.9 (0.5-7.3); n=5	**0.02**
Incidence of MRSA(95% C)I	2.7 (0.3-5.1); n=5	3.6 (0-8.5); n=2	2.3 (0-5); n=3	0.64
Incidence of *Streptococcus viridans* (95% C)I	7 (3.2-10.8); n=13	12.4 (3.2-21.6); n=7	4.7 (0.9-8.4); n=6	0.08
Incidence of CONS[3](95% CI)	4.3 (1.3-7.3); n=8	5.3 (0-11.4); n=3	3.9 (0.5-7.3); n=5	0.66
Incidence of *Enterococcus faecalis* (95% CI)	2.2 (0-4.3); n=4	3.6 (0-8.5); n=2	1.6 (0-3.7); n=2	0.39
Incidence of *Enterococcus brevis* (95% CI)	0.5 (0-1.6); n=1	1.8 (0-5.3); n=1	0 (0-0); n=0	0.17

N=total person-years, n= number of events, NA= not applicable, VRE[1] = Vancomycin-resistant *Enterococci*, MRSA[2] = Methicillin-resistant *Staphylococci*, CONS[3] = Coagulase-negative *Staphylococci*,*p = HIV-infected vs HIV-uninfected, **Incidence per 100 child years

Table 29: Incidence (per 100 child years) Gram-Negative Bacteria Causing Sepsis in HIV-Infected and HIV-Uninfected Children treated for Non-Hodgkin's Lymphoma

Gram-negative Pathogen	Overall N=185	HIV-Infected N=56	HIV-Uninfected N=128	p-value*
Incidence** of *Klebsiella* species (including ESBL[1]) (95% CI)	9.7 (5.2-14.2); n=18	12.4 (3.2-21.6); n=7	8.5 (3.5-13.6); n=11	0.45
Incidence of ESBL *Klebsiella* species (95% CI)	7.6 (3.6-11.5); n=14	10.7 (2.1-19.2); n=6	6.2 (1.9-10.5); n=8	0.33
Incidence of *Acinetobacter baumanni* (95% CI)	5.4 (2.1-8.8); n=10	10.7 (2.1-19.2); n=6	3.1 (0.1-6.2); n=4	**0.049**
Incidence of *Escherichia coli* (including ESBL) (95% CI)	4.9 (1.7-8); n=9	8.9 (1.1-16.7); n=5	3.1 (0.1-6.2); n=4	0.11
Incidence of ESBL *Escherichia coli* (95% CI)	1.1 (0-2.6); n=2	3.6 (0-8.5); n=2	0 (0-0); n=0	0.05
Incidence of *Pseudomonas aeruginosa* (95% CI)	4.9 (1.7-8); n=9	10.7 (2.1-19.2); n=6	2.3 (0-5); n=3	**0.022**
Incidence of *Enterobacter cloacae* (including ESBL) (95% CI)	3.8 (1-6.6); n=7	5.3 (0-11.4); n=3	3.1 (0.1-6.2); n=4	0.48
Incidence of ESBL *Enterobacter cloacae* (95% CI)	3.8 (1-6.6); n=7	5.3 (0-11.4); n=3	3.1 (0.1-6.2); n=4	0.48
Incidence of *Stenotrophomonas maltophilia* (95% CI)	3.2 (0.6-5.8); n=6	8.9 (1.1-16.7); n=5	0.8 (0-2.3); n=1	**0.006**
Incidence of *Bacillus* species (95% CI)	2.7 (0.3-5.1); n=5	7.1 (0.1-14.1); n=4	0.8 (0-2.3); n=1	**0.018**
Incidence of *Salmonella* species (including ESBL) (95% CI)	1.6 (0-3.5); n=3	5.3 (0-11.4); n=3	0 (0-0); n=0	**0.015**
Incidence of ESBL *Salmonella* species (95% CI)	1.6 (0-3.5); n=3	5.3 (0-11.4); n=3	0 (0-0); n=0	**0.015**

Incidence of *Aeromonas hydrophilia* (95% CI)	1.6 (0-3.5); n=3	1.8 (0-5.3); n=1	1.6 (0-3.7); n=2	0.91
Incidence of *Chromobacterium* (95% CI)	1.1 (0-2.6); n=2	0 (0-0); n=0	1.6 (0-3.7); n=2	0.61
Incidence of *Morganella morganella* (95% CI)	0.5 (0-1.6); n=1	1.8 (0-5.3); n=1	0 (0-0); n=0	0.17
Incidence of *Proteus mirabilis* (95% CI)	0.5 (0-1.6); n=1	0 (0-0); n=0	0.8 (0-2.3); n=1	0.87
Incidence of *Neisseria* species (95% CI)	0.5 (0-1.6); n=1	1.8 (0-5.3); n=1	0 (0-0); n=0	0.17
Incidence of *Bordetella* (95% CI)	0.5 (0-1.6); n=1	1.8 (0-5.3); n=1	0 (0-0); n=0	0.17

N=total person-years, n= number of events, NA= not applicable, ESBL[1] = Extended Spectrum β-lactamase producer, *p= HIV-infected vs HIV-uninfected, **Incidence per 100 child years

7.5.8. Fungi Associated with Sepsis in HIV-Infected and HIV-Uninfected Children treated for Non-Hodgkin's Lymphoma

Overall, there were 10 fungal MCSE per 100 child years. The most frequently identified fungal isolates were *Candida albicans* (n= 9; 52.9%), and *Candida parapsilosis* (n=3; 17.6%). The overall incidence (per 100 child years) of *Candida albicans* was five, and two for *Candida parapsilosis*. There were no significant differences in incidence of fungaemia between HIV-infected and HIV-uninfected children; Table 30.

7.5.9. Outcome

Of the 30 HIV-infected children, ten (33.3%) died because of sepsis. Of these, 60% had Stage 4 disease and the rest had Stage 3 disease.in addition, 80% of those who died had a CD4+ < 15%, and 70% had a HIV-viral load greater than 200 000 RNA copies/ml. Multiple pathogens were isolated from children who died including multi-drug resistant bacteria. Pneumonia was documented in 90% of children at the time of death; Table 31.

Table 30: Fungi Associated with Sepsis in HIV-Infected and HIV-Uninfected Children Treated for Non-Hodgkin's Lymphoma

Fungi	Overall N=185	HIV-Infected N=56	HIV-Uninfected N=128	P*-value
Incidence** of *Candida albicans* (95% CI)	4.9 (1.7-8); n=9	7.1 (0.1-14.1); n=4	3.9 (0.5-7.3); n=5	0.37
Incidence of *Candida parapsilosis* (95% CI)	1.6 (0-3.5); n=3	1.8 (0-5.3); n=1	1.6 (0-3.7); n=2	0.91
Incidence of *Cryptococcus* (95% CI)	1.1 (0-2.6); n=2	3.6 (0-8.5); n=2	0 (0-0); n=0	0.05
Incidence of *Candida tropicalis* (95% CI)	0.5 (0-1.6); n=1	1.8 (0-5.3); n=1	0 (0-0); n=0	0.17
Incidence of *Candida glabrata* (95% CI)	0.5 (0-1.6); n=1	0 (0-0); n=0	0.8 (0-2.3); n=1	0.87
Incidence of *Crytosporidium* (95% CI)	0.5 (0-1.6); n=1	0 (0-0); n=0	0.8 (0-2.3); n=1	0.87

N=total person-years, n= number of events, NA=not applicable, p*= Haematological malignancy vs Solid tumour, Incidence** per 100 child years

Table 31 Summary of Sepsis-Related Deaths in Children with Non-Hodgkin's Lymphoma

Number	Sex	Age (months)	CD4 (%)	CD4+ count	HIV-1 Viral load	Stage of NHL[1]	Blood Culture	Clinical Syndrome
1	Male	188	14.3	413	1 200	4	CONS[2], *Streptococcus viridans, Enterococcus faecium,* VRE[3], *Pseudomonas aeruginosa, Candida albicans*	Pneumonia, proven TB[6], GE[7], Herpes stomatitis, proven fungus
2	Male	171	8.7	119	320 000	4	CONS, *Bacillus* species, *Candida albicans*	Pneumonia, GE, proven fungus
3	Male	155	4.9	42	80 000	3	CONS, MRSA[4], *P. aeruginosa*	Pneumonia, Herpes stomatitis, meningitis
4	Female	146	3	275	224 000	4	Negative	Pneumonia, Herpes stomatitis
5	Male	132	19.4	220	300 000	4	ESBL[5] *Enterobacter cloacae*	Pneumonia, Herpes stomatitis
6	Female	82	6.2	57	618 000	3	Negative	Pneumonia, measles
7	Male	51	3.5	130	750 000	4	*Escherichia coli*	Herpes stomatitis
8	Male	50	7.9	331	15 000	4	Negative	Pneumonia, Herpes stomatitis
9	Male	40	12.3	630	3 000 000	3	ESBL *Salmonella*	Pneumonia
10	Male	19	26.5	613	458 000	3	ESBL *Klebsiella pneumonia*	Pneumonia, TB, Herpes stomatitis

NHL[1] =Non-Hodgkin's lymphoma, CONS[2] =Coagulase-negative *Staphylococci*, VRE[3] =Vancomycin-resistant *Enterococc*i, MRSA[4] =Methicillin-resistant *Staphylococc*i, ESBL[5] =extended spectrum β-lactamase producer, TB[6] =Tuberculosis, GE[7] =Gastroenteritis

7.6. DISCUSSION

In our study, underlying HIV-infection, male sex and age < 5 years were identified as independent risk factors for SSE and MCSE. However, although MCSE was 2.2 fold greater among HIV-infected children, it was not identified as an independent risk factor contributing to mortality in our study. The higher case fatality ratio (33.3%) in HIV-infected than HIV-uninfected children in our study was likely due to HIV-infected children more likely to presenting with Stage 4 NHL disease, and a high proportion of them being severely immunocompromised upon presentation.

Notably in our study, only one child of the thirty was known to be HIV-infected and on ARVs prior to the diagnosis of NHL. In the other 29, the HIV-infection was diagnosed concurrently with the malignant disease and was the AIDS defining presenting illness.

Diamond et al compared the survival of HIV-infected adults with NHL according to those who were diagnosed with HIV-infection and on ARVs prior to the diagnosis of NHL to those individuals whose HIV-infection was diagnosed concurrently with NHL and ARV naïve. The median survival in the group who were diagnosed with HIV-infection and NHL concurrently, was three months(Diamond et al., 2006).

There were no significant differences in the median age of presentation between HIV-infected (6.5 years) and HIV-uninfected children (4.8 years) in our study. Our cohort was, however, younger than reported in other studies among HIV-uninfected children with NHL, which have a reported median age of 8-10 years at presentation (Ahmad et al., 2010, Budiongo et al., 2015).

The majority of HIV-infected children in our study were severely immunosuppressed; with the median CD4+ percentage markedly low, including CD4+ percentage being < 15% in 63.3% of the children. This is similar to adults, among whom severe immunosuppression, defined by a low CD4+ count and percentage, was strongly associated with an increased systemic NHL rate in HIV-infected adults (Bower et al., 2009). However, adult patients in Africa have been also documented to present with HIV-associated NHL at a higher CD4+ count compared to those in HICs (Ulrickson et al., 2012).

All HIV-infected children and 86% of HIV-uninfected children in our study cohort, presented with advanced disease (stage 3 and 4). Advanced stage of presentation of NHL has been documented in LMICs (Ahmad et al., 2010) and in Africa (Budiongo et al., 2015) and in HIV-infected individuals (Gopal et al., 2012).

There were no significant differences in anthropometry in either group, except the children in the HIV-infected group were stunted (p=0.033). There are conflicting reports on the association of HIV-infection and stunting in children. One study reports no association with stunting in HIV-infected children (Sunguya et al., 2011) and another reports a prevalence of 68% (Nalwoga et al., 2010).

Both cohorts had leukopenia and pancytopenia at the time of the septic episode. The duration of neutropenia and lymphopenia during a septic episode was longer in the HIV-infected cohort. Prolonged neutropenia during a SE increased the odds of microbiologically confirmed sepsis.

HIV-infection is associated with haematological abnormalities, the most common manifestation being characterized by a reduction in, and the impaired function of all blood cell lines: anaemia, neutropenia, and thrombocytopenia (Kyeyune et al., 2014). However, in HIV-infected individuals, it is not only the virus but also the ARVs that both contribute to persistent hematopoietic suppression and ensuing cytopenias (Koka and Reddy, 2004).

The HIV-infected cohort had more suspected septic episodes (170 vs 80 per 100 child years), more microbiologically confirmed septic episodes (123 vs 48 per 100 child years), more episodes of Gram-positive bacteraemia (121 vs 35 per 100 child years), and more episodes of Gram-negative bacteraemia (102 vs 31 per 100 child years) than those in the HIV-uninfected cohort. We found the total number of pathogens from the blood, and the total number of bacteria in blood; and, the total number of pathogens and bacteria per septic episode was increased in the HIV-infected child. The most frequently isolated Gram-negative pathogens in our cohort were *Klebsiella* species, *Escherichia coli* and *Acinetobacter baumanni*, and the most common Gram-positive pathogens were *Streptococcus* species, *Enterococcus faecium*, and *Staphylococcus aureus*. None of the bacterial cultures isolated *Streptococcus pneumonia*. Gram-positive and Gram-negative bacteraemia increased the risk of all-cause mortality in our cohort.

Bacterial infections are a major source of morbidity and mortality in HIV-infected children (Jaspan et al., 2008). HIV-infected children have a greater risk of BSIs than their HIV-uninfected counterparts, and these infections are more invasive, more likely to disseminate, and have worse outcomes in HIV-infected children. In Europe and the United States, as well as in Africa, the main pathogen isolated from HIV-infected individuals without underlying malignancies is *Streptococcus pneumoniae* (22.8–58.5%). In our study cohort, we did not isolate *Streptococcus pneumoniae* in either group. In Africa, non-typable *Salmonella* is the most prominent isolate in non-cancer HIV-infected children (Huson et al., 2014). In addition, HIV-infected children often have multiple diagnoses and polymicrobial infections (McNally et al., 2007). Reported factors associated with BSIs in HIV-infected patients are related to the level of immunosuppression. In contrast, Musiime et al found no difference in CD4+ counts between HIV-infected non-cancer children with or without BSIs (Musiime et al., 2013).

Multi-resistant bacteria are reported to be more frequent in HIV-infected children (Cotton et al., 2008, Groome et al., 2012). We identified more ESBL *Escherichia coli* (p=0.05) and more ESBL *Salmonella* (p=0.015) in our HIV-infected cohort. However, Musiime et al reported a low prevalence of antimicrobial resistance in bacterial isolates from HIV-infected children in Uganda and Zambia (Musiime et al., 2013). There are no published data on HIV-infected children with Non-Hodgkin’s lymphoma; therefore, we are citing studies in HIV-infected non-cancer children.

We found a significantly increased presence of pneumonia ($p<0.001$) in the HIV-infected cohort. Pneumonia is common in HIV-uninfected children with cancer and a strong predictor of mortality (Bakhshi et al., 2008, Ghosh et al., 2012). There are no data published on the prevalence and outcome of pneumonia in HIV-infected children with cancer.

Fourteen of the 30 HIV-infected children had TB ($p=0.001$), and the odds of developing TB in the HIV-infected cohort was 19.2 times that of the HIV-uninfected cohort. HIV-infection may explain the frequency of TB in the HIV-infected child. HIV alters the pathogenesis of TB, and increases the risk of developing active TB in those with latent infection as well as in those newly exposed to TB. Corticosteroids are used in all cycles of chemotherapy for NHL. The increased immunosuppression due to corticosteroid administration could be associated with increased toxicity in patients with HIV due to other infectious comorbidities. The increased risk of re-activation or infection with MTB most likely explains the high prevalence in the HIV-infected cohort. In the HIV-uninfected population, only around 10% of people infected with TB will develop TB disease. However, in HIV-infected individuals, there is a 20-30 fold increased relative risk of developing TB disease from latent state compared with that in HIV-uninfected individuals . Key factors for the increased susceptibility of HIV-infected patients to develop TB include general as well as *Mycobacterium tuberculosis* specific CD4+ cell depletion (Zumla et al., 2013) and in the HIV-infected cohort, the use of high-dose corticosteroids also increases the susceptibility of TB re-activation. However, studies have also shown that HIV-infected individuals in high TB incidence regions have an increased risk of developing active TB. In addition, HIV-infected individuals on ARVs with high CD4+ counts continue to have an increased risk of developing TB (Gupta et al., 2012a, Venturini et al., 2014). This may be due to functional immune defects that may persist despite excellent CD4+ cell count recovery (Lawn et al., 2005). Another possibility is that individuals living with HIV in our community have greater exposure to TB or other common risk factors for the acquisition of both infections.

Ten (33.3%) of the HIV-infected cases and 3.6% of HIV-uninfected patients died due to sepsis in our study ($p=0.0006$). Other studies have also documented high case fatality ratio between 7-46% in non-cancer HIV-infected children with blood stream infections (Huson et al., 2014).

In the general population, the main risk factor for death is the HIV viral load. The odds of dying is almost 12 times ($p=0.014$) for those with log (VL) ≥ 4 compared to those with log (VL) < 4. The HIV viral load, after adjusting for CD4+ count, increases the risk of death. For each $\log_{10}$ increase in viraemia copy-years, the risk of death is increased by 44% (Mugavero et al., 2011) .

Although ARVs have reduced the mortality and morbidity of HIV-infected patients and lowered the incidence of AIDs- related cancers, lymphomas remain a major issue in the HIV-infected population. The main concerns regarding the use of chemotherapy in HIV-infected patients, compared with the general population, are subsequent immune depletion and an increased rate of infections, mostly because, like most NHLs, AIDS-related cancers generally occur when the CD4+ count is low and the patient is severely immunosuppressed. As children with HIV survive longer due to better antiretroviral and supportive therapies, HIV-related malignancies may become an increasingly common problem.

All children in the study presented with advanced stage of NHL. The factors associated with delay in diagnosis of cancers in children are complex. In children with HIV-infection, TB and cancer, the diagnosis is complicated by the similarities in clinical presentation. Current recommendations include greater awareness of HIV-infection, regular clinical evaluation of HIV-infected children paying particular attention to the warning signs of cancer (Poyiadjis et al., 2011). Pneumonia and TB are major complications in the HIV-infected child treated for NHL. In LMICs, ventilator support is minimal and this lack contributes to death in this cohort of children. All HIV-infected children with NHL should be screened for TB and TB treatment should be initiated at the earliest opportunity. It has not been the practise to treat this high-risk group of children with TB prophylaxis in our setting. This may be an important protocol to develop for the future.

HIV-infected children with NHL have a high bacterial load complicating septic episodes. Many studies have reported on the use of prophylactic antibiotics in the HIV-uninfected adults with cancer; there is a higher risk of antimicrobial resistance in adults given prophylactic antibiotics. In South Africa, multi-resistant bacterial isolates from children infected with HIV, is a growing concern (Groome et al., 2012). The use of prophylactic antibiotics will add to the burden.

The overall burden of cancer is a growing problem in LMICs who are faced with the difficult choices of allocation of scarce financial resources for cancer awareness, screening, diagnosis, and treatment of the disease and supportive. Cancer is often a low public health priority, such that treatment approaches take precedence over prevention, early detection, and palliative care. As a result of these policies, most cancer patients in developing countries are diagnosed with late-stage disease (Jones et al., 2006).

The cost of cancer care is a barrier to increasing access in LMICs. Non-communicable diseases are a global health and economic threat; and “cancer now kills more people each year in LMICs than AIDS, tuberculosis, and malaria combined” (Jones et al., 2006). This highlights the ethical and financial imperative to improve the care of lymphoma globally and serves as a call to action for the haematology- oncology community (Ulrickson et al., 2012).

8. Thesis Conclusion

Infectious complications following treatment in the Black South African child with cancer is a major burden, which contributes to morbidity and mortality in this group of children. Our study demonstrated the incidence (per 100 child years) of suspected septic episodes was 103, and microbiologically confirmed sepsis 96.7, both of which were higher in children with haematological malignancies (HM) than in solid tumours (ST). Blood cultures were positive in 71% of suspected septic episodes. We also recorded that 51.1% of microbiologically confirmed sepsis was due to Gram-positive bacteraemia, 40.4% to Gram-negative bacteraemia, 8.5% to fungaemia, and all were more common in the cohort with haematological malignancies than solid tumours. Each patient experienced multiple septic episodes during the course of treatment, 4.02 in those with HM and 2.3 in those with ST. There were also polymicrobial septic episodes seen in both cohorts. The incidence (per 100 child years) of Gram- positive bacteraemia, Gram-negative bacteraemia, and fungaemia was 58.9, 47.9, and 9.2, respect-ively, all of which were also higher in children with HM. The most frequently isolated Gram-positive bacterial pathogens were Coagulase-negative *Staphylococci, Streptococcus viridans,* and *Enterococcus faecium*; Gram-negative pathogens were *Escherichia* species, *Acinetobacte*r species, and *Klebsiella* species, and fungi were *Candida albicans* and *Candida parapsilosis*. Studies from other LMICs have documented MSCE ranging from 25-50%, Gram-positive bacteraemia accounting for 20-60%, and Gram-negative bacteraemia, from 50-60% of MSCE (Gupta et al., 2011a, Bakhshi et al., 2008).

The high incidence of sepsis in our study could be due to many of the patients presenting with advanced and aggressive disease, including 25% of children with ST and 61% of those with NHL presenting with Stage 4 disease and 78% of acute lymphoblastic leukaemia patients categorized as having high-risk disease. The presentation with such advanced disease, as well as the related high intensity chemotherapy regimens, advanced and aggressive surgery, and wide field radiotherapy, which is required to manage such children, was independently associated with sepsis in our study. The cohort with high-risk HM had a 4.01 greater risk of microbiologically confirmed sepsis than those with medium-risk HM. Treatment intensity category 2 and 3 was associated with a 2.03 and 8 fold greater risk of microbiologically confirmed sepsis than those who received treatment intensity 1. In addition, children with metastatic ST had a 2.49 fold higher risk of microbiologically confirmed sepsis than those with localized disease. In addition, although our study did not identify malnutrition to be an independent risk factor for sepsis, the majority of patients were malnourished, including 76.9% by MUAC and 56.8% by arm muscle area.

The HIV-infected cohort had more suspected septic episodes (170 vs 80 per 100 child years), more microbiologically confirmed septic episodes (123 vs 48 per 100 child years), more episodes of Gram-positive bacteraemia (121 vs 35 per 100 child years), and more episodes of Gram-negative bacteraemia (102 vs 31 per 100 child years) than those in the HIV-uninfected cohort.

We found a significantly increased presence of pneumonia ($p<0.001$) in the HIV-infected cohort. Ten (30%) of the HIV-infected cases and 3.6% of HIV-uninfected patients died due to sepsis in our study Fourteen of the 30 HIV-infected children had TB ($p=0.001$), and the odds of developing TB in the HIV-infected cohort was 19.2 times that of the HIV-uninfected cohort.

The complexity of manging children with malignancies for sepsis in our setting, including the high (40.6%) of children in whom multiple pathogens were identified for an individual sepsis episode. In addition, we documented a high prevalence of antibiotic resistance for some pathogens, including 65% of *Enterococcus faecium* being resistant to Vancomycin, > 85% resistance to penicillin and 62% *Enterococcus faecium* strains being resistant to Ampicillin. Furthermore, 25% of *Staphylococcus aureus* strains were methicillin-resistant. The majority of Gram-negative pathogens were, however, susceptible to the Carbapenems except for *Acinetobacter baumanni* and *Pseudomonas aeruginosa*. More than 50% of *Escherichia coli and* Klebsiella *pneumoniae* were resistant to the third generation Cephalosporins. *Pseudomonas aeruginosa* isolates showed less resistance to the antimicrobials tested than most of the other Gram-negative pathogens, and *Acinetobacter baumanni* was resistant to most antimicrobials tested.

All deaths related to sepsis occurred in children with HM, and more with high-risk HM than those with medium-risk HM. Contributing factors to death in our study included treatment with high-dose steroids and chemotherapy treatment intensity. Additionally, although sepsis itself was not identified as an independent risk factor for death, the CFR was greater in children who developed pneumonia and in those with microbiologically confirmed TB. In addition, among children who died due to sepsis, the odds of dying were greater in those who had profound neutropenia and lymphopenia at the onset of sepsis, and those who had prolonged neutropenia and lymphopenia during the course of a septic episode. Children who are at risk for profound and prolonged pancytopenia should, if possible, be isolated and carefully monitored for the earliest signs of sepsis.

The incidence of tuberculosis in our study cohort was 4.7 per 100 child years (6.8 in HM vs 2.9 in ST), including 50% of tuberculosis cases being microbiologically confirmed among whom usually only 10-30% of TB cases are microbiologically confirmed. Nevertheless, we showed that tuberculin skin test and the IGRA assay were poorly sensitive for diagnosing underlying MTB infection, with only 2.9% of the children having a reactive TST. This would have been expected to be closer to 15-18% at 6-7 years of age (mean age group in our study), based on the annual risk for MTB infection in children being 3% year-on-year (Wood et al., 2011)

There were a high percentage of respiratory viral associated septic episodes in our patients. The overall incidence (per 100 child years) was 92.4 in those with HM, and 33.1 in those with ST. The most frequently isolated viruses were human Rhinovirus, Coronavirus, and human Polyomavirus. 68.3% of respiratory viral associated septic episodes had one virus isolated, 22.2% had two viruses, and 10.4% had more than two viruses per septic episode. Overall, 22.4% of septic episodes had bacterial/fungal and respiratory viral co-infections, 33.9% in those with HM, and 22.7% in those with ST. The viruses most frequently associated with co-infections were yet again human Rhinovirus, Coronavirus, and human Polyomavirus.

Respiratory viruses were associated with 66.3% of pneumonia cases, and the most commonly isolated were HRV, CV HUK1, and KIPyV. Influenza A virus was documented in

9.8% of pneumonia, and 2.6% of non-pneumonia cases. The exact causal role of respiratory viruses to sepsis in children is unclear.

In our study cohort, 53% of patients died. Of these, 13 (7.6%) were related to sepsis. All of those who died had HM, and all four Non-Hodgkin's lymphoma patients who died, were HIV-infected. The cohort who died had polymicrobial infections with bacteria (including multi-drug resistant Gram-positive and negative bacterial pathogens), fungi, and respiratory viruses. Influenza A virus was identified in two patients at the time of death. It would be prudent to consider vaccinating children with cancer with the Influenza A vaccine seasonally.

Awareness and education of children's cancer, improved primary health care facilities for cancer screening and detection, and prompt referral to specialist centres will result in earlier diagnosis and improved survival in children's cancer, which reaches cure rates (with bone marrow transplantation) of up to 80% in HICs.

Non-communicable diseases are a global health and economic threat; and "cancer now kills more people each year in LMICs than AIDS, tuberculosis, and malaria combined" (Jones et al., 2006). It is urgent and imperative to improve the awareness, early detection, and optimize cancer and supportive care of lymphoma and other malignant diseases and serves as a call to mobilize health authorities and the haematology-oncology community to reduce the morbidity and mortality of this curable disease (Ulrickson et al., 2012)

The major issues that need further clarification in our cohort of children is the relationship between polymicrobial sepsis and disease severity and outcome, the causal role of respiratory viral infections in children with cancer, and their association with death. Ideally, these patients should have post-mortem studies to help delineate the cause of death. We also need to be more vigilant with the early identification of tuberculosis, and to plan a trial of Isoniazid prophylaxis in high-risk cancer children. Influenza A virus was identified in two children who died, which may have been prevented by vaccination. Influenza immunization, if possible, should be given seasonally to children receiving anti-cancer therapy. The paediatric-oncology community should add their voice to the early detection and treatment of HIV-infected children. Additionally, the warning signs of cancer should be widely disseminated with the hope of early detection of childhood cancers (Poyiadjis et al., 2011).

9. APPENDICES

9.1. Appendix 1: Admission Data

Patient number	Study number
Date of birth	Date of presentation
Sex	RTHC
Diagnosis 1=ALL, 2=AML, 3=NHL, 4=HD, 5=WT, 6=RBL, 7=NBL, 8=OS, 9=BT, 10=RMS, 11=HBL, 12=HCC, 13=GCT, 14=KS, 15=NPC, 16=other	
PPD 1=yes, 2=no	BCG scar
Blood tests	
WCC	Hb
MCV	PLT
Na K	Urea creatinine albumin
HIV 1=pos, 2=neg Date of Dx	IgG IgM IgA
Fe transferrin	TB Ellispot
CXR	
Mother: highest level of education	Mother: Employment Status

9.2. Appendix 2: Anthropometry on Admission

Pt ID	Study Number
Date of Birth	
Date of Presentation	
Height in cm	
Weight in kg	
MUAC in mm	
TSFT in cm	

9.3. APPENDIX 3: CLINICAL HISTORY FOR SEPSIS ADMISSION

- Previous documented infections and colonization
- Stage of disease and treatment
- Remission status
- Non-infectious cause of fever e.g. transfusion of blood products
- Underlying co-morbid conditions e.g. recent surgery
- Use of corticosteroids
- Presence of indwelling catheters
- Duration of fever
- Site specific symptoms: myalgia, headache, cough, vomiting, diarrhoea, dysuria, perianal pain or bleeding, chest symptoms, ear pain, sore throat, skin lesions
- Use of granulocyte colony stimulating agents
- Radiotherapy (dates, dose, field)

9.4. Appendix 4: Physical Examination for Sepsis Admission

- Mucous membranes for mucositis , bleeding, fungal infections, herpes infection
- Dentition for periodontal abscesses
- Sinuses
- Oropharynx
- Lungs
- Abdomen for pain
- Perineum for signs of infection
- CNS: level of consciousness, meningism, intra- cranial bleeding.

9.5. Appendix 5: Admission Data for Sepsis

Study Number		Sepsis number	
Chemo Protocol		Start Date End Date	
Date of fever	T	T max	Duration of T > 38°C
BP			
WCC			
Neutrophil count			
Lymphocyte count			
Monocyte count			
Plt Plt T/f (y/n) Number of units		HB PC T/f (y/n) Number of units	
GCSF y/n Duration		U/E	
CRP		PCT	
BDG		LFT	
Blood Culture		Urine Culture	
Sputum for AFB		Other Site Culture	
Antibiotics		Antibiotic Duration	
CXR		Clinical Syndromes	
Respiratory Virus y/n			
Outcome of Sepsis			

9.6. APPENDIX 6: RESPIRATORY VIRUSES

- Respiratory Syncytial Virus
- Influenza A
- Influenza B
- Parainfluenza 1, 2, 3
- Human Rhinovirus
- Human Metapneumovirus
- Human Bocavirus
- Coronavirus 229E, OC43, NL 63, HUK1
- Adenovirus
- Human Polyomavirus WUPyV, KIPyV

9.7. APPENDIX 7: ANTIBIOTIC PROTOCOL

9.7.1. INITIAL ANTIBIOTIC PROTOCOL

- Piperacillin- Tazobactam (300 mg/ kg divided into 3 doses) i.v.i.
- Amikacin (15 mg/ kg daily) i.v.i.
- Vancomycin (40 mg /kg in 2 divided doses) i.v.i. will be added if the patient has an indwelling venous access device (central line/ or Portocatheter) or if the patient is hemodynamically unstable**.**

9.7.1.1. Modification of Therapy in the First Week of Treatment

9.7.1.1.1. Patient becomes afebrile in 3 – 5 days

If an aetiologic agent is identified, therapy will be adjusted to the most appropriate antibiotic(s). If no aetiologic is identified, the initial antibiotics will be continued. If the blood cultures remain negative for gram-positive organisms, vancomycin will be discontinued (if already commenced).

9.7.1.1.2. Persistent Fever throughout the First 3-5 Days

Therapy will be reassessed on day 3. If there is no clinical worsening, the initial antibiotics will be continued. If there is persistent fever, the initial antibiotics will be stopped, a septic workup repeated and Meropenem (40 mg/ kg/ dose 8 hourly) i.v.i will be added.

If the patient is febrile after 5 days, Amphotericin B (1 mg/ kg daily) i.v.i. will be added.

9.7.2. DURATION OF ANTIBIOTICS

9.7.2.1. Patient is Afebrile by Day 3

If the patient's neutrophil count is greater than or equal to 500 cells/µL for two consecutive days, if there is no definite site of infection and if cultures do not yield positive results, antibiotic therapy will be stopped when the patient is afebrile for more than or equal to 48 hours. If the patient's neutrophil count is < 500 cells/µL by day seven, if there are no subsequent complications antibiotic therapy will be stopped when the patient is afebrile for five-seven days.

9.7.2.2. Persistent Fever on Day 3 of Antibiotics

If the patient's neutrophil count is ≥ 500 cells/µL, stop antibiotic therapy four-five days after the neutrophil count is greater than or equal to 500 cells/µL. If the patient's neutrophil count is < 500 cells/µL, reassess and continue antibiotic therapy until the neutrophil count is ≥ 500 cells/µL.

9.7.3. Use of Antiviral Drugs

Acyclovir (15 mg / kg per dose 8 hourly) i.v.i or Acyclovir p.o. will be commenced and the choice of intravenous or oral will be determined by the severity of the infection

9.7.4. Use of Colony – Stimulating Factors

Colony stimulating factors i.e. granulocyte colony stimulating factors (G-CSF) will be used as per chemotherapy protocol.

9.7.5. Antibiotic Prophylaxis

Trimethoprim-sulfamethoxazole will be used to prevent *Pneumocystis jirovecii* in high–risk patients.

9.7.6. Selective Gut Decontamination

Patients presenting with diarrhoea will be treated with Cholestyramine, Metronidazole, and, Gentamycin.

9.8. APPENDIX 8: ASSENT FORM

Good day, my name is Dr Naidu. I work in the Paediatric Department, Haematology Oncology unit, Chris Hani Baragwanath Academic Hospital

You have been diagnosed with cancer. Your treatment will include chemotherapy/radiotherapy/ surgery. Treatment for cancer weakens the immune system, which predisposes the patient to different forms of infections caused by bacteria, fungi, or viruses. Infections in patients with cancer can be very serious and therefore have to be treated aggressively.

You are invited to consider participating in a research study to determine the factors which predispose the patient with cancer to infections, the type of infection and how best to pick up the infections early and to treat them effectively. The total amount of time required for your participation in this study will be the duration of the cancer treatment and the follow-up period.

If you do agree to participate in the study, a medical history will be obtained, followed by a physical examination. Twenty mls i.e. four teaspoons of blood will be withdrawn from you for laboratory investigations. Drawing of the blood may result in faintness, inflammation of the vein, pain, bruising or bleeding. Your nutritional status will be assessed by blood tests and a DEXA scan.

You will not benefit directly from your participation in the study, but the medical knowledge acquired from the study may help other patients who have cancer in the future.

Participation in the study is voluntary and you can decline to participate, or stop at any time without stating any reason. Please inform me if you decide to discontinue participating in the study.

All information obtained during the course of the study, including hospital records and research data will be kept strictly confidential.

9.9. Appendix 9: Informed Consent

9.9.1. Introduction

Good day, my name is Dr Gita Naidu. I work in the Paediatric Department, Haematology Oncology Unit, Chris Hani Baragwanath Academic Hospital. Your child has been diagnosed with cancer and will receive chemotherapy/radiotherapy surgery and these forms of treatment may be complicated by infections.

You are invited to consider your child to participate in a research study to investigate the predisposing factors for infections in patients with cancer, the type of infections and to study the best way to pick up these infections early This will enable us to give the most effective form of treatment.

Your child's participation in this study is voluntary. Before agreeing for your child to participate, it is important that you read and understand the purpose of the study, the study procedures, risks and discomforts, and your right to refuse for your child to participate and to withdraw from the study at any time.

This information leaflet is to help you to decide on whether your child should participate in the study. If there are any questions, do not hesitate to ask me. You should not agree to participate in the study unless you properly understand all the procedures involved in this study. If you agree for your child to participate in this study, this document has to be signed to confirm an understanding of the study. You will be given a copy to keep.I would like to request permission to review your child's hospital records

9.9.2. Purpose of the Study

Your child has been diagnosed with cancer and I would like you to consider your child taking part in the research of the infections that may be experienced during the treatment. The purpose of this study is to determine the predisposing factors for infectious complications for children with cancer, to determine the spectrum and resistance patterns of infections and to determine the early markers of infections.

9.9.3. Duration of the Study and Number of Participants

The study will be performed at the. Chris Hani Baragwanath Academic Hospital. Approximately 400 participants will be enrolled in this study. The participants will be between the ages of 0 and 18. The total amount of time required for participation in this study will be the duration of the treatment of the cancer and the follow-up period.

9.9.4. Procedures

If you agree to allow your child to participate in this study, information about your child's health will be obtained followed by a physical examination. These are part of the standard routine for all patients admitted to the hospital. Before receiving treatment, blood for standard tests done as part of the hospital protocol for all patients with cancer plus 20 mls of blood (4 teaspoons) for study specific tests will be taken. A DEXA scan will be performed initially to document the participants nutritional status and thereafter at six monthly intervals. For each hospital admission for sepsis (approximately six times during treatment), blood tests which are done as part of the hospital protocol and X-rays will be performed. In addition, nasal swabs and bloods for nutritional and infective markers will be done as part of the study 20 mls i.e. 4 teaspoons).

9.9.5. Will Any of these Procedures Result in Discomfort or Inconvenience?

Drawing blood may result in faintness, inflammation of the vein, pain, bruising or bleeding at the puncture site. There is also a slight possibility of infection. Your protection is that experienced personnel perform the procedures under sterile conditions. A total of 100 ml of blood (i.e. half a cup) will be collected over the course of the entire study.A chest x-ray, a commonly used diagnostic procedure and a DEXA scan exposes the participant to a small amount of radiation. Although all radiation is cumulative over your lifetime, small doses should not create a significant risk to your health.

9.9.6. BENEFITS

There are no benefits to your child resulting from participation in the study. Participation in this study will contribute to medical knowledge that may help other patients who have cancer in the future. Rights as a participant in this study participation in this study is voluntary and you can decline to participate, or stop at any time, without stating any reason. Withdrawal will not affect access to other medical care. ***Please inform me if you decide to discontinue your child's participation in the study.***

9.9.7. ETHICAL APPROVAL

This clinical study protocol has been submitted to the University of the Witwatersrand, **Human Research Ethics Committee (HREC)** and written approval has been granted. The study has been structured in accordance with the **Declaration of Helsinki** (last updated: October 2000), which deals with the recommendations guiding doctors in biomedical research involving human participants. A copy may be obtained from me should you wish to review it.

9.9.8. SOURCE OF ADDITIONAL INFORMATION

For the duration of the study, you will be under my care. If at any time between your visits, you feel that any of your symptoms are causing you any problems, or you have any questions during the study, please do not hesitate to contact me. Other doctors from this department who are working on this study are: Dr L Wainwright, Dr S Poyiadjis, Dr D Mackinnon, and Dr B Rowe

The 24-hour telephone number for Dr G. Naidu is 082 788 8190.

If you want any information regarding your **rights as a research participant, or complaints regarding this research study**, you may contact Professor Cleaton-Jones, Chairperson of the University of the Witwatersrand, HREC, which is an independent committee established to help protect the rights of research participants at (011) 717 2229.10.8.

9.9.9. CONFIDENTIALITY

All information obtained during the course of this study, including hospital records, personal data and research data will be kept strictly confidential. Data that may be reported in scientific journals will not include any information that identifies you as a participant in this study. Any information uncovered regarding your test results or state of health as a result of your participation in this study will be held in strict confidence. You will be informed of any findings of importance to your child's health or continued participation in this study but this information will not be disclosed to any third party in addition to the ones mentioned above without your written permission. The only exception to this rule will be cases of communicable diseases where a legal duty of notification of the Department of Health exists. In this case, you will be informed of my intent to disclose such information to the authorised state agency.

9.9.10. INFORMED CONSENT FOR PARENTS/LEGAL GUARDIANS

(On behalf of minors under 18 years old)

Dr Gita Naidu has provided me with a copy of the Participant Information Leaflet and Consent regarding the study "Infectious Complications in African Children with Cancer" and has fully explained to me the nature, benefits, and purpose of the study. The study doctor has given me the opportunity to ask any questions concerning the study. It has been explained to me that I will be free to withdraw my child from the study at any time, without any disadvantage to future care. I have understood everything that has been explained to me and I consent for my child to participate in this clinical study.

PARENT/LEGAL GUARDIAN:

Printed Name	Signature/Mark or Thumbprint	Date and Time

PARTICIPANT ASSENT: * (Seven years old and above)

Printed Name	Signature/Mark or Thumbprint	Date and Time

(* Minors competent to understand must participate as fully as possible in the entire procedure)

STUDY DOCTOR:

Printed Name	Signature	Date and Time

TRANSLATOR/OTHER PERSON EXPLAINING INFORMED CONSENT …………….… (DESIGNATION):

Printed Name	Signature	Date and Time

WITNESS (If applicable)**:**

Printed Name	Signature	Date and Time

9.10. Appendix 10: Subject Information Sheet

Infectious Complications in African Children with Cancer

Hello! My name is Dr. Gita Naidu.

I am a Paediatric Oncologist working in the Paediatric Haematology-Oncology Unit, Chris Hani Baragwanath Academic Hospital, and University of the Witwatersrand.

Patients with cancer receive chemotherapy and / or radiotherapy which weakens the blood and predisposes them to infections, which may be life threatening. Malnutrition during treatment may increase their susceptibility to infections. Bacteria, viruses, fungi, or parasites may cause these infections.

Our present treatment is uniform irrespective of the cause of the infection and some patients may be over treated e.g. if viral infections are treated with antibiotics.

We want to carry out a study on the causes and predisposing factors to infections in children with cancer. We would also like to investigate the most reliable and earliest marker of infection to ensure the most effective means of treatment.

The blood and radiological investigations are those that are done routinely.

Nasopharyngeal aspirates will be collected to test for acute respiratory viruses.

All information obtained will remain confidential. Allocated study numbers will protect your anonymity.

9.11. Appendix 11: Berlin Frankfurt Munster Non-Hodgkin's Lymphoma 1995 Protocol

B-CELL NON-HODGKIN LYMPHOMA PROTOCOL

BFM-NHL 95

Risk category definitions

R1	Completely resected stage I and abdominal stage II
R2	Nonresected stage I/II and stage III with LDH< 500
R3	Stage III with LDH 500 – 999
	Stage IV, B-ALL (>25% blasts), no CNS disease and LDH < 1000
R4	Stage III, IV, B-ALL, and LDH ≥ 1000
	Any CNS disease

Risk groups and Therapy courses

R1	**A**	**B**					
R2	**V**	**A**	**B**	**A**	**B**		
R3	**V**	**AA**	**BB**	**CC**	**AA**	**BB**	
R4	**V**	**AA**	**BB**	**CC**	**AA**	**BB**	**CC**

***V = Prephase**

Conditions for starting second and subsequent blocks:

Platelets > 50 x 10^9/L and neutrophil counts higher than 0.5 x 10^9/L

Aim for 21 days between blocks

Groups R3 and R4 **:** **G-CSF 5µg/kg after first 2 therapy courses**

Groups R3 and R4: **Assessment CT after fifth course of therapy and manage as follows:**

If residual tumor present, to biopsy. If biopsy reveal residual viable tumor, megadose chemotherapy with ABSCT.

If no residual viable tumor, R4 group to receive last course CC, and R3 group to receive no further therapy.

PROTOCOL

PREPHASE (V)

Dexamethasone orally /IV	5mg/m² on days 1 and 2
	10 mg/m² on days 3,4,5
Cyclophosphamide IV over1hr	200mg/m² on days 1 and 2
Methotrexate IT	12mg on day 1
Cytarabine IT	30mg on day 1
Hydrocortisone IT	10mg on day 1

COURSE A

Dexamethasone orally/IV	10mg/m² in 3 div doses days 1 to 5
Vincristine IV	1.5mg/m² (max 2 mg) day 1
Ifosfamide IV over 1hr	800mg/m² days 1 to 5
Mesna	800mg/m² days 1 to 5
Cytarabine IV over 1hr	150mg/m² - 2 doses 12 hourly on days 4 and 5 *(total 4 doses)*
Etoposide IV over 1hr	100mg/m² on days 4 and 5
Methotrexate IV	**1g/m² over 4 hours on day 1**

** *Leucovorin rescue*

30mg/m² IV at hour 24 from start of Methotrexate infusion.

Thereafter 15mg/m² IV 6 hourly x 6 doses

Methotrexate IT	12mg on day 1
Cytarabine IT	30mg on day 1
Hydrocortisone IT	10mg on day 1

COURSE B

Dexamethasone orally /IV	**10mg/m^2 in 3 div doses days 1 to 5**
Vincristine IV	1.5mg/m^2 (max 2 mg) day 1
Cyclophosphamide IV over 1hr	200mg/m^2 days 1 to 5
Doxorubicin IV over 1 hr	25mg/m^2 days 4 and 5
Methotrexate IV	**1g/m^2 over 4 hours on day 1**

** *Leucovorin rescue*

30mg/m^2 IV at hour 24 from start of Methotrexate infusion.

Thereafter 15mg/m^2 IV 6 hourly x 6 doses

Methotrexate IT	12mg on day 1
Cytarabine IT	30mg on day 1
Hydrocortisone IT	10mg on day 1

COURSE AA

Dexamethasone orally/IV	10mg/m^2 in 3 div doses days 1 to 5
Vincristine IV	1.5mg/m^2 (max 2 mg) day 1
Ifosfamide IV over 1hr	800mg/m^2 days 1 to 5
Mesna	800mg/m^2 days 1 to 5
Cytarabine IV over 1hr	150mg/m^2 - 2 doses 12 hourly on days 4 and 5 (*total 4 doses*)
Etoposide IV over 1hr	100mg/m^2 on days 4 and 5
Methotrexate IV	**5g/m^2 over 24 hours on day 1**

** *Leucovorin rescue*

75mg/m^2 IV at hour 36 from start of Methotrexate infusion.

Thereafter 15mg/m^2 IV 3 hourly x 8 doses, 15mg/m^2 IV 6 hourly x 6 doses

Methotrexate IT	12mg on day 1 and 5
Cytarabine IT	30mg on day 1 and 5
Hydrocortisone IT	10mg on day 1 and 5

COURSE BB

Dexamethasone orally /IV	10mg/m² in 3 div doses days 1 to 5
Vincristine IV	1.5mg/m² (max 2 mg) day 1
Cyclophosphamide IV over 1hr	200mg/m² days 1 to 5
Doxorubicin IV over 1 hr	25mg/m² days 4 and 5
Methotrexate IV	**5g/m² over 24 hours on day 1**

** *Leucovorin rescue*

75mg/m² IV at hour 36 from start of Methotrexate infusion.

Thereafter 15mg/m² IV 3 hourly x 8 doses, 15mg/m² IV 6 hourly x 6 doses

Methotrexate IT	12mg on day 1 and 5
Cytarabine IT	30mg on day 1 and 5
Hydrocortisone IT	10mg on day 1 and 5

COURSE CC

Dexamethasone orally/IV	20mg/m² in 3 div doses days 1 to 5
Vincristine IV	1.5mg/m² (max 2mg) on day 1
Cytarabine IV over 3hrs	3g/m² - 2 doses 12hourly on days 1 and 2 (*total 4 doses*)
Etoposide IV over 2 hrs	100mg/m² - 12hourly doses on days 3, 4 and single dose on day 5 (*total 5 doses*)

Methotrexate IT	12mg on day 5
Cytarabine IT	30mg on day 5
Hydrocortisone IT	10mg on day 5

9.12. Appendix 12: Turn-It-In Document

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ORIGINALITY REPORT

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9.13. APPENDIX 13: FIRST LINE ANTIRETROVIRAL THERAPY

Age Group	Treatment
< 3 years <10 kg	Abacavir + Lamivudine + Lopinavir/Ritonavir
3-10 years > 10 kg	Abacavir + Lamivudine = Lopinavir/Ritonavir
10-15 years < 40 kg	Abacavir + Lamivudine + Efavirenz
≥ 15 years > 40 kg	Tenofivir + Lamivudine + Efavirenz

9.14. Appendix 14: Ethics Document

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Naidu

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M080304**
PROJECT	Infectious complications in African children with Cancer
INVESTIGATORS	Dr G Naidu
DEPARTMENT	Paediatrics
DATE CONSIDERED	08.03.25
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 08.06.23 **CHAIRPERSON** ....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof S Madhi

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

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